Soybean Genetics Newsletter

Volume 19 April 1992

Agricultural Research Service - USDA
Department of Agronomy
and Department of Genetics
Iowa State University
Ames, Iowa 50011

Table of Contents

FOREWORD

In 1990, we found it necessary to institute a subscription fee for the Soybean Genetics Newsletter. This year, we added a late fee for those subscribing after 1 January.

The continual success of this newsletter depends almost entirely upon you, the reader and contributor.

We call your attention to the Fourth Biennial CONFERENCE OF MOLECULAR AND CELLULAR BIOLOGY OF THE SOYBEAN to be held at Iowa State University, 27-29 July, 1992. For further information contact:

Dr. Randy C. Shoemaker
USDA ARS FCR
Dept. of Agronomy
Iowa State University
Ames, Iowa 50011
Telephone: 515-294-6233
FAX: 515-294-2299

Our sincere appreciation goes to Arricka Earp and her assistants, Holly Heer, Teresa Harper and Terry Couch, who did a lot of the detailed work that always accompanies any such project.

- R.G. Palmer, editor

Data presented in the Soybean Genetics Newsletter are not to be used in publication without the consent of the respective author.

Mention of a trademark or proprietary product by the USDA or Iowa State University does not imply its approval to the exclusion of other products that may also be suitable.

SOYBEAN GENETICS COMMITTEE REPORT

February, 1992

Minutes of the Meeting

The Soybean Genetics Committee met from 7:30 p.m. to 8:00 p.m. on Monday, February 17, at the Sheraton Plaza Hotel in St. Louis, Missouri, in conjunction with the Soybean Breeders' Workshop.

Committee members attending the meeting were: G. R. Buss, R. I. Buzzell, P. B. Cregan, T. C. Kilen, S. A. Mackenzie, R. L. Nelson, C. D. Nickell, R. G. Shoemaker, and J. R. Wilcox. P. B. Cregan and C. D. Nickell had been elected by mail ballot to serve a three-year term on the Committee. At the conclusion of the meeting C. D. Nickell was elected Chairman for the year ending in February, 1993.

Also in attendance at the meeting were Claudia Coble, J. H. Orf, and J. E. Specht. Current Committee members and the February expiration dates for their terms on the Committee are:

C. D. Nickell, (1995) Chairman
Turner Hall - Agronomy
1102 South Goodwin
University of Illinois
Urbana, Illinois 61801
(217)-333-9461
FAX (217)-333-9817

G. R. Buss (1993)
Crop, Soil, and Environ. Sci. Dept.
Virginia Polytechnic Institute
and State University
Blacksburg, Virginia 24061
(703)-231-9788
FAX (703)-231-3431

R. I. Buzzell (1994)
Crop Science Section
Agriculture Canada Research Station
Harrow, Ontario NOR 1GO, Canada
(519)-738-2251
FAX (519)-738-2929

P. B. Cregan USDA-ARS (1995)
Nitrogen Fixation and Soybean
Genetics Laboratory
Bldg. 011, BARC West
Beltsville, Maryland 20705
(301)-504-5070
FAX (301)-504-5728

S. A. Mackenzie (1993)
Department of Agronomy
Purdue University
1150 Lilly Hall of Life Sciences
West Lafayette, Indiana 47907-1150
(317)-494-6380
FAX (317)-494-6508

R. L. Nelson USDA-ARS (ex officio)
Turner Hall - Agronomy
1102 South Goodwin
University of Illinois
Urbana, Illinois 61801
(217)-244-4346
FAX (217)-333-4639

R. G. Palmer USDA-ARS (ex officio)
Departments of Agronomy and Genetics
Iowa State University
Ames, Iowa 50011
(515)-294-7378
FAX (515)-294-2290

R. C. Shoemaker USDA-ARS (1994)
Department of Agronomy
Iowa State University
Ames, Iowa 50011
(515)-294-6233
FAX (515)-294-2299

Procedure: As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols, and linkages should be sent to the chairman of the Soybean Genetics Committee for review. To facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." Manuscripts will not be reviewed for style except as this influences the clarity of interpretation. Manuscripts will not be "peer reviewed" unless requested by the author. Authors may submit unpolished (but comprehensible) manuscripts for review, unless peer review is requested. This should reduce delays involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of three weeks to return the reviewed manuscript to the Chairman (who will then give it to the author as soon as possible). If the reviewers have not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman of the Soybean Genetics Committee.

Assignment/Approval of Gene Symbols: If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is Curator for all maturity groups.

Gene symbols will be approved only in cases where the relevant material is available in one of the soybean germplasm collections for distribution to researchers. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretation does not depend upon symbols, in most cases.) The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is not necessary.

Summaries for the Past Year: A list of the soybean gene symbols and linkages approved during the year March 1991 through February 1992 is given in Table 1.

Committee Actions: A Committee of R. C. Shoemaker (Chairman), P. B. Cregan, and J. E. Specht was formed to designate standard nomenclature for assignment of RFLPs and to explore the possibilities of a standard reference map for soybean. This committee will submit a report to the Chairman of the Soybean Genetics Committee by June 1, 1992.

All future correspondence should be addressed to C. D. Nickell, Chairman, Soybean Genetics Committee.

J. R. Wilcox
Past Chairman

Table 1. Gene symbols and linkage groups approved, March 1991-February, 1992.

Date	Authors	Trait/Linkage	Gene/Linkage	Genetic Type
May 31, 1991	Palmer and Groose	Dilute-purple flower	*w4-dp*	T321
June 10, 1991	Buzzell and Anderson	Resistance Pmg 16,18,19	*Rps7*	-
		Linkage Group 10	*Rps7*-12.5-*Rps1*	-
Aug. 23, 1991	Amberger et al.	Aconitase null	*Aco2-bn*	T318
Aug. 29, 1991	Lohnes and Bernard	Powdery mildew resistance	*Rmd-c*	-
Sept. 20, 1991	Palmer et al.	Linkage group 20	*Rxp*-15.81-*Mdh*	-
Dec. 30, 1991	Stephens and Nickell	Pink flower	*wp*	-
Dec. 30, 1991	Buzzell and Buttery	Anomalous flavonol glycoside	*Fg2-a*, *Fg2-b*	-
Dec. 31, 1991	Hedges and Palmer	Malate dehydrogenase	*Mdh1-n* (Urbana) *y20* (Urbana)	T253
			Mdh1-n (Ames 1) *y20* (Ames 1)	T317
			Mdh1-n (Ames 2) *y20* (Ames 2)	T323
			Mdh1-n (Ames 3) *y20* (Ames 3)	T324
			Mdh1-n (Ames 4) *y20* (Ames 4)	T325

1992 SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee (CAC) held its annual meeting February 17, 1992 at the Soybean Breeders Workshop in St. Louis, Missouri. Thirteen of the fourteen members were in attendance. Those elected to three year terms were Dr. John Hicks, Pioneer Hi-Bred International, Greenville, MS, representing private breeding - South.; Dr. Fritz Schmitthenner, Ohio State University, Wooster, Ohio, representing plant pathology; and Dr. Dennis Egli, University of Kentucky, Lexington, KY, representing plant physiology for a second consecutive three year term. Dr. Schmitthenner was in attendance as well as Mike Kenty, Agronomist, USDA, Stoneville, MS; Dr. Dick Buzzell, Public Breeder, invited observer from Canada; Mark Bohning, GRIN, USDA, Beltsville, MD.

Dr. Roger Boerma called the meeting to order and instructed the three Soybean CAC subcommittees of Acquisition, Evaluation and Operations to discuss issues and bring recommendations to the entire committee. Subcommittees were as follows:

Acquisition: H.R. Boerma, W.J. Kenworthy, R.L. Nelson and J.G. Shannon; Evaluation: T.S. Abney, D.B. Egli (absent), T.C. Kilen, L. Lambert and L.D. Young; Operations: C.J. Coble, E.E. Hartwig, T.E. Devine, J. Thorne and P.A. Miller.

Claudia Coble reported on the current status of the soybean germplasm collection and reviewed 1991 seed distribution. There were 5623 and 7870 accessions sent to the US and foreign countries, respectively. Distributions by the state and foreign country for various institutions were presented in a handout. There are about 13,600 Glycine max strains in the collection. Evaluation data have been summarized for PI 490.765 through PI 507.573 (Groups 000 to IV) and the manuscript for a USDA Technical Bulletin has been submitted for publication. Tentative agreement has been reached with the Chinese Institute of Crop Germplasm Resources to acquire 500 germplasm accessions (Group IV and earlier) in the Spring of 1992. If the current agreement is maintained, 500 more accessions will be received from Southern China in two years (Group V and later).

The number of accessions in Glycine soja is currently over 1000. The current inventory of the USDA Perennial Glycine Germplasm collection consists of 849 accessions representing 15 species. The USDA Southern and Northern Germplasm collections were consolidated into a single collection in February, 1991. This transition through the cooperative efforts of the northern and southern curators has gone smoothly.

Mark Bohning gave a GRIN progress report. There are currently 700 users with access codes. He reported on a new centralized computer system with much more storage and faster turnaround on jobs. Software is being written to be more user friendly and to include new sources of data including isozymes, RFLPs, gene mapping studies and international databases. There is a possibility that additional databases such as animal, insect microbial, fish, etc., will be housed on the new system. Germplasm requests can be made directly on GRIN without typed or written requests. Updates are being made on older PI's to get the same information on GRIN as the newer accessions.

Subcommittee reports given

Acquisition Subcommittee: Randy Nelson reviewed the policy of the USDA Soybean Germplasm Collection regarding Crop Science Registered Germplasm Releases. This policy has been approved by Crop Science. Segregating populations will be deposited in the National Seed Storage Laboratory (NSSL) but not held in the Collection at Urbana. All soybean breeders and geneticists will be encouraged to publish segregating germplasm releases as a Note in Crop Science rather than a registration article. Soybean breeders and geneticists and appropriate administrators in various institutions will be notified of this policy. Each releasing institution is to take official responsibility for maintaining and distributing seed of all their Crop Science registered segregating material for a minimum of five years.

Soybean germplasm lines that are registered in Crop Science will be deposited in the NSSL and the collection at Urbana, IL. The collection will store, distribute and increase these lines as necessary. Every line will be reviewed ten years after

registration by the committee. If the progenitors of the line are maintained in the collection and the committee can find no compelling reason to continue to maintain the line, it will be removed from the collection, but will remain in the NSSL.

Roger Boerma addressed the need to send the policy to ESCOP to inform them so they can officially act on their responsibility to maintain segregating germplasm for a specified period. Randy Nelson made the motion (seconded by Tom Kilen) to send the policy to ESCOP regarding Crop Science Registered Germplasm Releases, but that the policy be in agreement with the actual consolidated policy set forth by Crop Science concerning releases of segregating germplasm and originators' responsibility to maintain the releases for a specified period. The motion passed.

Evaluation Subcommittee: Lavone Lambert reported that Groups V-X have been screened for resistance to soybean looper. Fifteen additional genotypes have been identified with as much resistance as the three original genotypes (PI 227.687, PI 229.358 and PI 171.451). All data are being put in GRIN. He reported that the soybean germplasm Groups V-X will be screened for resistance to velvetbean caterpillar with completion in 1994.

Lawrence Young completed screening newer PI's in Groups V-X to soybean cyst nematode races 3, 5 and 14, starting where Sam Anand left off. He found three PI's resistant to race 5 but susceptible to the other races. Newer accessions in Group 00 to IV starting where Dr. Anand left off are being screened to races 3, 5 and 14 with completion expected by the end of 1992. All of these results will be added to GRIN.

Scott Abney reported screening some Group III and IV accessions from certain geographic areas plus other widely used accessions with resistance to other pathogens to Sudden Death Syndrome (SDS). Athow and Laviolette data accumulated for Phytophthora screening are being added to GRIN.

Tom Kilen reported that Bob Keeling, USDA Pathologist, Stoneville, MS screened 50 Group V and 165 Group VI accessions to stem canker. Resistant lines had a level of resistance similar to Tracy M with 41% and 58% of the Group V and Group VI,

respectively, showing resistance. These results will be added to GRIN.

The large number of accessions sent out, but return of little useful data was addressed. Ways were discussed to encourage feedback on evaluation to retrieve useful data to be put on GRIN. A motion was made by Tom Kilen and seconded by Lavone Lambert that the expertise of the evaluation subcommittee be used by the curators to encourage feedback to retrieve data from germplasm evaluations. The motion passed.

Operations subcommittee: Claudia Coble reported that there are seven publications concerning the soybean germplasm collection. She indicated that the curators are considering a yearly Soybean Collection Newsletter to report unusual characteristics that are found and what is going on with the collection including new evaluations and where evaluations are being conducted. Randy Nelson called for ideas on how to continuously update lists without publishing entire lists.

An update on germplasm research cooperation with China was given. The USDA-ARS, the Illinois Agricultural Experiment Station, the Illinois Soybean Program Operating Board, the Iowa Soybean Promotion Board, and the Iowa Agricultural and Home Economics Experiment Station have contributed funds to support a germplasm exchange and evaluation project with China. The plan is for a Chinese scientist to bring germplasm to the US for this cooperative research. The first exchange of germplasm will be accessions from areas of northern china that are poorly represented in the current USDA germplasm collection. Plans are being made for a second shipment containing accessions from southern China. Future cooperative projects are possible if the needed financial support can be arranged.

Dr. Hartwig asked what will be done with the data in the three-year evaluation of latest varieties exchanged between the US and China with common tests conducted in each country. Randy reported we have received only one year's data from China. He thought that they had only conducted the tests two years instead of three. It was suggested that the three years data from the US

be put in a publication.

Roger Boerma expressed concern on getting nominations for the Soybean CAC from Plant Physiologists, Plant Pathologists and Entomologists without official mailing lists for each group. It was suggested that current members of the Soybean CAC come up with nominations and formalized mailing lists for various disciplines.

Elections were held for the positions of Chairman and Vice-Chairman. Roger Boerma, University of Georgia and Tom Devine, USDA, ARS, Beltsville, were elected Chairman and Vice-Chairman, respectively.

There being no other business the meeting was adjourned.

Roger Boerma, Chairman
Soybean Germplasm Crop Advisory Committee

USDA Soybean Germplasm Collection Report

February 1992

In 1992, a total of 7,870 seedlots were distributed from the USDA Soybean Germplasm Collection in response to 323 requests from 38 states and 27 foreign countries. Seed orders were placed by 265 US requestors for 5,623 seedlots and by 58 foreign requestors for the remaining 2,247. Numerous publications were sent in response to 19 requests for information about the collection. Additionally, 749 accessions have been packeted to send to the National Seed Storage Laboratory at Ft. Collins, CO as back-up samples for the collection. This is part of an ongoing project to increase the seed sample size to 1,500 seeds for all accessions at the National Seed Storage Laboratory.

Of the approximately 13,600 Glycine max strains in the Collection in 1991, 730 were grown in Stoneville, MS and 1,462 were grown in Urbana, IL for seed replacement. After being purelined in 1991, approximately 127 new G. max lines were added to the collection and are now available for distribution. These additions are from the USSR, China, Nepal, Japan, and Taiwan. The 165 maturity group X accessions are currently being purelined at the Tropical Agricultural Research Station, Isabela, Puerto Rico with the assistance of Salvio Torres and Nabor Mendoza. By growing two generations per year, the purelining will be completed in August 1992. In October 1992 we will plant the 153 maturity group IX accessions plus approximately 200 new introductions received in 1991 from Tom Rulkin, Malang Research Institute for Food Crops, East Java, Indonesia.

Evaluation data has been summarized for PI 490.765 through PI 507.573 (maturity groups 000 to IV) and the manuscript for a USDA Technical Bulletin has been submitted for publication. This bulletin will be ready for distribution this spring. This year we will be beginning a general evaluation of all accessions in maturity groups V through VIII at Stoneville. In 1992-93, approximately 800 accessions in maturity group VI will be evaluated. During and immediately after these evaluations extra

seed will be available from these lines for other research projects. If anyone would like access to this seed please contact either Tom Kilen at Stoneville, MS or Randall Nelson or Claudia Coble at Urbana, IL. Forty-eight germplasm maintenance plots of Glycine soja were grown in 1991. Of the 100 new lines added to the wild soybean collection 1991, 30 were from China and the remainder were from the USSR. The current inventory of the USDA Wild Soybean Germplasm Collection now exceeds 1000 accessions. Two new accessions of wild soybean from China were grown for the first time in 1991. The Glycine soja at Urbana are all grown inside aphid-proof cages. This allows for greater seed production, better expression of morphological characters and should significantly reduce the virus infection of the seed.

In 1991, 154 new accessions were grown for the first time. These originated from the USSR, Japan, and mostly from China. The following institutions donated germplasm to our collection this year: Botanical Gardens, Department of Genetics, Voronezh State University, Voronezh, Russia; National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan; Department of Agriculture, Animal Husbandry and Fishery, Heilongjiang Province, China; Asian Vegetable Research and Development Center, Taiwan; Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, Beijing, China; Heilongjiang Academy of Agricultural Sciences, Harbin, Heilongjiang China; Soybean Research Institute, and Nanjing Agricultural University, Nanjing, Jiangsu, China. The following US scientists helped to obtain accessions, and their assistance is greatly appreciated: M. Rangappa, Virginia State University; T. Carter, USDA-ARS, North Carolina State University, and J. Konovsky, Washington State University and T. Hymowitz, University of Illinois.

New G. max accessions received to date that will be planted in 1992 include accessions from Japan, China, Vietnam, and the former USSR.

A report on the USDA Perennial Glycine Germplasm Collection is given by Ted Hymowitz in a separate article in this volume.

The USDA Southern and Northern Soybean Germplasm collections were consolidated into a single collection in February 1991. Tom Kilen and his research group at Stoneville, MS are growing all the accessions in maturity groups V through VIII, but all seed requests should to be addressed to the USDA Soybean Germplasm Collection at the address given at the end of this article.

The present inventory of G. max and G. soja by sub-collection and maturity group is a follows:

	000	00	0	I	II	III	IV	V	VI	VII	VIII	IX	X	Total
Pre-1945 public cultivars	3	5	7	23	26	38	38	9	16	19	18	0	0	202
Post-1945 public cultivars	1	18	26	38	56	48	50	23	23	20	9	3	0	315
Private cultivars	0	0	0	6	5	6	9	0	0	0	2	0	0	28
Clark isolines	0	0	0	0	0	0	274	0	0	0	0	0	0	274
Harosoy isolines	0	0	0	0	117	0	0	0	0	0	0	0	0	117
Williams isolines	0	0	0	0	0	62	0	0	0	0	0	0	0	62
Other isolines	0	0	0	10	10	17	0	0	0	0	0	0	0	37
Genetic types	0	1	1	15	33	40	49	3	0	3	1	0	0	146
FC accessions (G. max)	1	4	6	3	6	13	18	10	10	17	2	0	0	90
PI accessions (G. max)	106	386	882	1,176	1,349	1,252	2,618	1,795	765	409	328	153	165	11,384
PI accessions (G. soja)	76	70	58	55	65	24	82	322	163	79	1	3	3	1,001
Totals:	187	484	980	1,323	1,668	1,502	3,138	2,162	977	547	361	159	168	13,656

C.J.Coble and R.L. Nelson
University of Illinois
USDA-Agricultural Resource Service
1102 S.Goodwin Avenue
Urbana, Illinois 61801

CANADA

UNIVERSITY OF GUELPH
Crop Science Department
Guelph, ON N1G 2W1 Canada
and
AGRICULTURE CANADA
Research Station
Harrow, ON N0R 1G0 Canada

1) Soybean linkage tests

Linkage tests were conducted with two disease resistance loci, Rps1 rps1 and Rps6 rps6, and two herbicide reaction loci, Hb hb and Hm hm. Crosses of 'Altona' and 'MiB' with 'Kentland' were assayed for linkage among a number of traits. The differing alleles for the traits are given in Table 1 for the three parents. MiB is a line selected from Misaodaizu x Harosoy (Rennie et al., 1992).

Flavonol classes were determined by thin-layer chromatography using leaf samples from F_2 plants. Flower and pubescence color were rated on F_2 plants or their progenies. Hilum color of F_2 and F_3 seeds was recorded. Seedcoat peroxidase was determined using seeds from F_2 plants. Isoenzymes were asssayed using starch gel electrophoresis of samples taken from the cotyledons of young F_2 seedlings. Race 1 of Phytophthora megasperma f.sp. glycinea was used to screen for Rps1-c and Rps6 using hypocotyl wounding/mycelium insertion. Bentazon and metribuzin reaction was tested using applications of the herbicides in separate fields. Seed lots of about 100 F_4 seeds each were composited by picking up to 33 three-seeded pods from each $F_{2:3}$ row for use in the disease and herbicide tests.

Rps1 was not linked with Enp (Table 2). Other tests (Rennie et al., 1987a; 1987b; 1987c) did not detect linkages between the Rps1 and Rps6 loci and the Aco4, Enp, and Idh2 loci. The results allowed us to test Rps1 and Hm(LG10) with several markers from other linkage groups, since this has not been done. Hm is not

linked with R(LG2), I(LG7) or Ep (LG12) and Rps1 is not linked with Ep (Tables 3 and 4). Buzzell (1979) showed that Rps1 was not linked to T and Fg3(LG1), L1(LG5) and W1(LG8).

There is no indication of any close linkages among the loci tested (Tables 2, 3 and 4). Rps6 might be loosely linked (40.6 ± 5.7) with Fg2(P=0.04) and will need to be re-tested. The probability of 0.01 for the Rps6-Hm combination suggests linkage, but there is an excess of the non-parental types, indicating that linkage is unlikely. Using the product method(Immer and Henderson 1943), calculations indicate the Rps6 and Hm are independent.

B.D. Rennie
R.I. Buzzell
T.R. Anderson

References

Buzzell, R.I. 1979. Soybean linkage tests. Soybean Genet. Newsl. 6:15-16.

Immer, F.R. and M.T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Rennie, B.D., W.D. Beversdorf and R.I. Buzzell. 1987a. Assaying for linkage between Rps and isozyme loci in soybean. Crop Sci. 97:519-521.

___, ___, ___. 1987b. Genetic and linkage analysis of an aconitate hydratase variant in the soybean. J. Hered. 78:323-326.

___, ___, ___. 1987c. Inheritance and linkage analysis of two endopeptidase variants in soybeans. J. Hered. 78:327-328.

___, R.I. Buzzell, T.R. Anderson and W.D. Beversdorf. 1992. Evaluation of four Japanese soybean cultivars for Rps alleles conferring resistance to Phytophthora megasperma f. sp. glycinea. Can. J. Plant Sci. 72: In press.

Suiter, K.A., J.F. Wendel and J.S. Case. 1983. LINKAGE-1: A Pascal computer program for the detection and analysis of genetic linkage. J. Hered. 74:203-204.

Table 1. Alleles for which Altona and MiB differed from Kentland

Trait	Altona	MiB	Kentland
Pmg reaction	Rps6	Rps1-c	rps1rps6
Bentazon response		hb	Hb
Metribuzin response	hm	hm	Hm
Flavonol glycoside	Fg2fg3	Fg2fg3	fg2Fg3
Seedcoat peroxidase	Ep	Ep	ep
Flower color	W1		w1
Pubescence color	T		t
Aconitase	Aco4-b		Aco4-a
Endopeptidase	Enp-a	Enp-a	Enp-b
Isocitrate dehydrogenase		Idh2-a	Idh2-b
Hilum color	R		r
Hilum color		I	i^i

Table 2. Isoenzyme linkage tests

Loci pair	(LG)	Aa B_	AA B_	aa B_	Aa bb	AA bb	aa bb	N	R	SE	P*
		MiB X Kentland									
Aco4 - Fg2		63	30	40	23	11	14	181	49.7	4.6	0.99
Aco4 - Fg3	(,1)	66	33	45	19	8	10	181	48.3	4.6	0.82
Aco4 - Hb		62	42	32	21	8	13	178	43.4	4.5	0.30
Enp - Fg2		81	27	33	29	8	13	191	47.4	4.4	0.86
Enp - Fg3	(,1)	92	25	31	20	9	14	191	46.6	4.4	0.16
Enp - Ep	(,12)	80	27	37	28	8	11	191	49.7	4.4	0.89
Enp - I	(,7)	76	24	32	31	11	12	181	48.6	4.5	0.92
Enp - Rps1	(,10)	85	27	35	23	8	10	188	49.3	4.5	0.98
Enp - Hb		81	40	26	25	7	8	187	44.3	4.4	0.45
Idh2 - Fg2		80	34	34	23	15	15	201	50.0	4.3	0.41
Idh2 - Fg3	(,1)	83	36	38	17	12	12	198	49.4	4.4	0.43
Idh2 - Ep	(,12)	74	36	40	30	15	10	205	45.6	4.2	0.45
Idh2 - Hb		75	39	40	28	9	11	202	48.8	4.3	0.48
		Altona x Kentland									
Aco4 - Fg2		58	34	28	24	13	10	167	49.2	4.7	0.94
Aco4 - Fg3	(,1)	69	38	27	13	9	12	168	43.0	4.6	0.16
Aco4 - Hm	(,10)	67	38	32	16	9	7	169	50.0	4.7	0.98
Enp - Fg2		72	24	32	25	11	9	173	45.5	4.6	0.64
Enp - Fg3	(,1)	78	25	32	20	10	9	174	46.3	4.6	0.61
Enp - Ep	(,12)	82	25	30	19	11	12	179	49.2	4.6	0.24
Enp - Hm	(,10)	77	29	29	23	7	13	178	44.0	4.5	0.46

LG = Linkage Group

'A' refers to the Aco4-a Enpa or Idh2-a allele, 'a' refers to the Aco4-b Enp-b or Idh2-b allele.

'B' refers to the Fg2, Fg3, Ep, I, Rps1-c, T, Hm or Hb allele, 'b' refers to the fg2, fg3, ep, i, rps1, t, hm or hb allele.

* Chi-square probability with 2 df (Suiter *et al.* 1983) for independence; expected ratio 6:3:3:2:1:1.

Table 3. Other Rps linkage tests

Loci pair	(LG)	Allele combinations[+] a	b	c	d	N	R	E	P*	Phase
		MiB x Kentland								
Rps1 - I	(10,7)	121	44	31	13	209	48.3	5.3	0.70	C
Rps1 - Hb	(10,)	125	42	35	10	212	47.8	5.3	0.68	R
Rps1 - Ep	(10,12)	125	44	34	11	214	48.7	5.2	0.83	C
		Altona x Kentland								
Rps6 - Fg2		121	40	30	20	211	40.6	5.7	0.04	C
Rps6 - Fg3	(,1)	126	34	43	11	214	49.2	5.2	0.89	R
Rps6 - T	(,1)	120	39	36	18	213	44.0	5.5	0.21	C
Rps6 - Ep	(,12)	120	45	45	11	221	44.0	5.4	0.26	C
Rps6 - Hm	(,10)	136	37	28	19	220	37.8	5.7	0.01	R
Rps6 - W1	(,8)	118	39	38	13	208	49.5	5.2	0.93	C
Rps6-R	(,2)	89	36	29	10	164	47.6	6.0	0.70	C

LG = Linkage Group
[+] a=XY, b=Xy, c=xY, and d=xy for the dominant and recessive alleles in each loci pair.
* Chi-square probability with 1 df (Suiter *et al.* 1983) for independence; expected ratio 9:3:3:1.

Table 4. Other linkage tests for herbicide reaction genes

Loci pair		Allele combinations a	b	c	d	N	R	SE	P*	Phase
		MiB x Kentland								
Hb - Fg2		114	44	42	10	210	43.2	5.6	0.22	R
Hb - Fg3	(,1)	124	34	39	13	210	47.2	5.3	0.60	C
Hb - I	(,7)	120	38	32	19	209	41.4	5.7	0.07	R
Hb - Ep	(,12)	114	40	36	14	204	48.6	5.3	0.78	R
Hb - Hm	(,10)	109	48	36	15	208	49.2	5.2	0.88	C
Hm - Fg2		106	37	46	17	206	49.0	5.3	0.87	R
Hm - Fg3	(10,1)	115	29	47	16	207	46.6	5.4	0.40	C
Hm - I	(10,7)	104	39	44	18	205	48.3	5.3	0.80	R
Hm - Ep	(10,12)	111	35	45	19	210	45.8	5.4	0.38	R
		Altona x Kentland								
Hm - Fg2		120	50	34	11	215	45.9	5.3	0.51	R
Hm - Fg3	(10,1)	136	33	32	13	214	42.3	5.5	0.17	C
Hm - T	(10,1)	128	41	29	16	214	42.8	5.5	0.13	R
Hm-W1	(10,8)	124	41	32	11	208	49.4	5.2	0.92	R
Hm - Ep	(10,12)	132	41	32	15	220	44.4	5.3	0.25	R
Hm - R	(10,2)	90	39	25	7	161	43.2	6.3	0.35	R

LG = Linkage Group
+a = XY, b = Xy, c = xY, and d = xy for the dominant and recessive alleles in each loci pair.
*Chi-square probability with 1 df (Suiter et al. 1983) for independence; expected ratio 9:3:3:1.

CHINA

SOYBEAN INSTITUTE
JILIN ACADEMY OF AGRICULTURAL SCIENCES
Gongzhuling, 136100
Jilin Province
The People's Republic of China

1) Effect of Cercospora sojina Hara on lipid peroxidation in soybean plants

Introduction: Cercospora sojina Hara is a major disease of soybean production in most regions of China. The injury to plants has become more serious in recent years, thus there is a great deal of attention directed toward this disease. There were some investigations about the screening and identification of resistant genotypes, biochemical characters and the inheritance of resistance to C.sojina Hara. Less information is available about the change of metabolism in soybean plants when they are injured by C.sojina Hara. The purpose of this study was to attempt to analyze the relationship between some biochemical characters related to lipid peroxidation and resistant mechanisms of soybean plants to C.sojina Hara using genotypes with different resistant levels.

Materials and methods: Three resistant and three susceptible lines were selected to use in this experiment based on the result of artificial inoculate treatment. The experimental C.sojina Hara was collected in different regions of Jilin province and provided by Hejiang Institute of Agricultural Sciences.

The plants were potted on July 3, 1991. There were four replications for each treatment and four plants for each pot. The plants were transferred to an inoculate pond and inoculated with C.sojina separate compound when the third trifoliolate was fully expanded.

*This project supported by NNSF of China

The plants were covered immediately to avoid light and to keep humidity for 24 hours. Most of the plants exhibited disease symptoms after 18 days. The inoculated and uninoculated middle leaflets were taken simultaneously for biochemical analysis when the plants exhibited disease symptoms for two days.Each assay consisted of three replications.

Assay of SOD activity followed the method described by Shao et al. (1983). Determination of POD activity followed the method of Mead (1976). Assay of CAT followed the method of Shao (1983). Determination of chlorophyll and ascorbic acid followed the methods in Handbook of Plant Physiology (1980, 1987).

Results: 1) Results shown in Fig. 1-a indicated that the activity of SOD in soybean leaves increased when the plants were injured by Cercospora sojina Hara for both resistant and susceptible lines, and the activity of susceptible lines was higher than that of resistant lines at the same time; 2) The change of POD activity was similar to that of SOD activity. It is shown in Fig. 1-b that the POD activity increased, and the activity of susceptible lines was higher than that of resistant lines after the plants were injured by C.sojina Hara; 3) Levels of ascorbic acid in soybean leaves decreased for both resistant and susceptible lines when the plants were injured by C.sojina Hara, and the content of ascorbic acid of susceptible lines was higher than that of resistant lines both before and after the plants were injured by C.sojina Hara, (Fig. 1-c). The CAT activity and chlorophyll content also were analyzed in this experiment, but there were no obvious trends that can be seen (Fig. 1-d,e).

Discussion: Three enzymes, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), were chosen for investigation in this experiment. Their combined effects are known to reduce the concentration of O_2-, OH-, and H_2O_2-, products of the partial reduction of oxygen, in living plants. Thus they are expected to limit the production of free radicals which are known to cause membrane degradation through lipid peroxidation (Fridovich, 1978; Mead, 1976).

It can be seen from this study that the activity of SOD and POD increased and the level of ascorbic acid decreased as the plants were injured by Cercospora sojina Hara. This probably means that membrane damage occurs during this period, and the change of SOD, POD and ascorbic acid may be a reaction of the soybean plant to C.sojina Hara. The high level of SOD and POD activities may be essential for survival of injured plants, and the unequal response of the various biochemical characters to C.sojina Hara may be regarded as a possible mechanism facilitating survival during this period.

References:

Fridovich, I. 1975. A Rev. Biochem. 44:147-159.
___, 1978. Science. NY 201:875-880.
He Zhaofan. 1985. Techniques of food and oil quality analysis. Agri. Press. Pp:210-213.
Huadong Normal Uni. 1980. Handbook of plant physiology. People's Education Press. Pp:143-144.
Mead, J.F. 1976. In "Free radicals in biology". Academic Press. NY Pp:51-68.
Shandong Agri. College. Handbook of plant physiology. Shanxi Science & Technology Press. Pp:47-48.
Shao Chengben. 1983. Plant physiology communications. 5:46-49.
Xibei Agri. Uni. 1987. Handbook of plant physiology. Shanxi Science & Technology Press. Pp:47-48.

Zhuang Bingchang
Liao Lin
Wang Yumin
Liu Yuzhi
Xie Xueju
Xu Bao

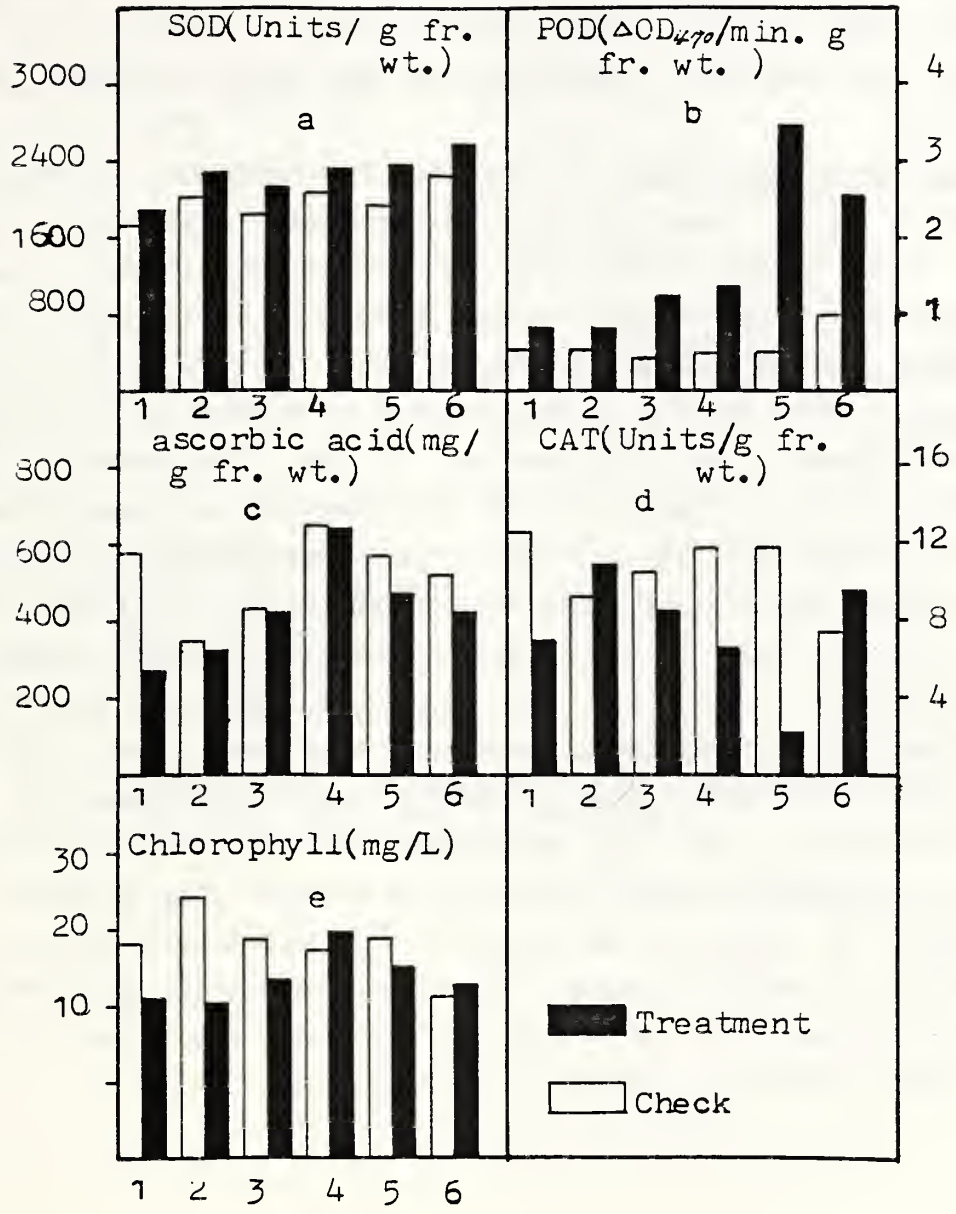


Fig. 1 Change of some biochemical characters in soybean leaves when the plants were injured by Cercospora sojina Hara

1, 2, 3: resistant lines

4, 5, 6: susceptible lines

2) A new electrophoretic variant of SBTi-A2 in soybean seed protein

The Kunitz trypsin inhibitor was found in 1945 in soybean seed protein. It was designated SBTi-A2 through electrophoresis and genetic study by Singh et al. (1969), Hymowitz, Orf, Kaizuma (1972, 1973, 1978, 1979, 1981). There were three alleles in this locus, named Ti^a, Ti^b, and Ti^c, separately. The null genotype (ti) was also found.

Materials and methods: There were 243 samples, collected from northwestern China, used in this experiment. Extraction of SBTi followed the method modified by Hymowitz (1972), and the electrophoresis followed the method modified by Hu et al. (1983). SBTi markers were a product of Sigma Co.

Results: One SBTi-A2 electrophoretic variant was found in 243 soybean germplasms from northwestern China. The band of SBTi of this germplasm is different in Rf from marker and genotypes which were known as Ti^a, Ti^b, Ti^c. This variant was determined ten times, and the results coincided. The Rf for Ti^c is 0.87, Ti^a is 0.85, Ti^b is 0.82, and new type is 0.79 in our laboratory conditions.

Discussion: There was only one new genotype found in the 11,081 Chinese soybean germplasms we examined. It might be a fourth allele of SBTi. The evidence was as follows: 1) it was different from Ti^a, Ti^b, Ti^c genotypes and markers. 2) the band (Rf=0.79) disappeared when the loading sample, the trypsin, and a new compound of trypsin and trypsin inhibitor was found in the region near the negative pole. This is similar to the results of marker plus trypsin. 3) no other protein band was found in the new band region in other germplasms we examined.

A new electrophoretic variant of SBTi-A2 in soybean (G.max) seed protein

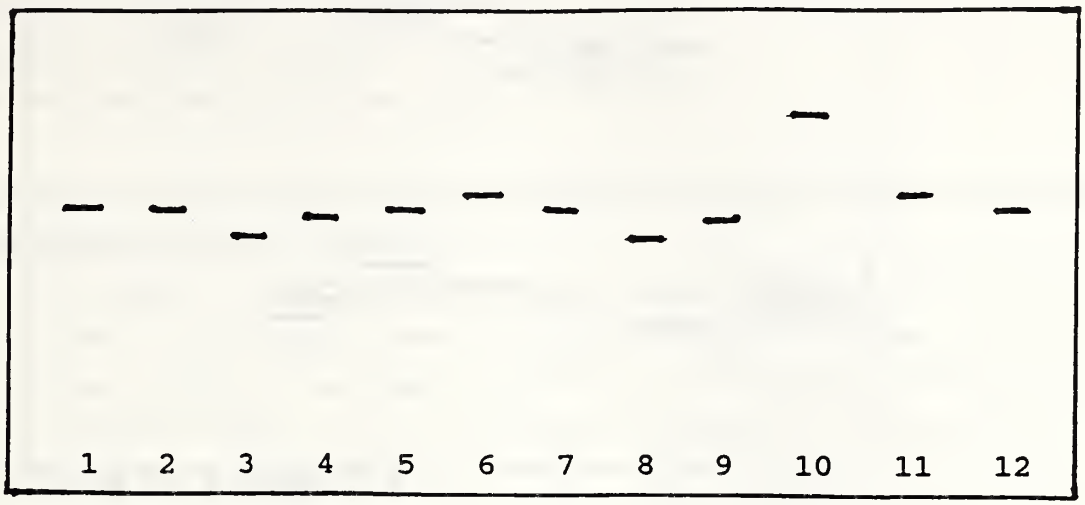


Figure 1

1, 2, 7, 12: Sigma Co. marker;
3: $\underline{Ti}^{c}$ genotype;
4: $\underline{Ti}^{a}$ genotype;
5, 8: $\underline{Ti}^{b}$ genotype;
6, 9: SBTi new genotype;
10: New genotype plus trypsin, band above "*" is the compound of trypsin and trypsin inhibitor;
11: $\underline{Ti}^{a}$, $\underline{Ti}^{b}$, $\underline{Ti}^{c}$, and a new band.

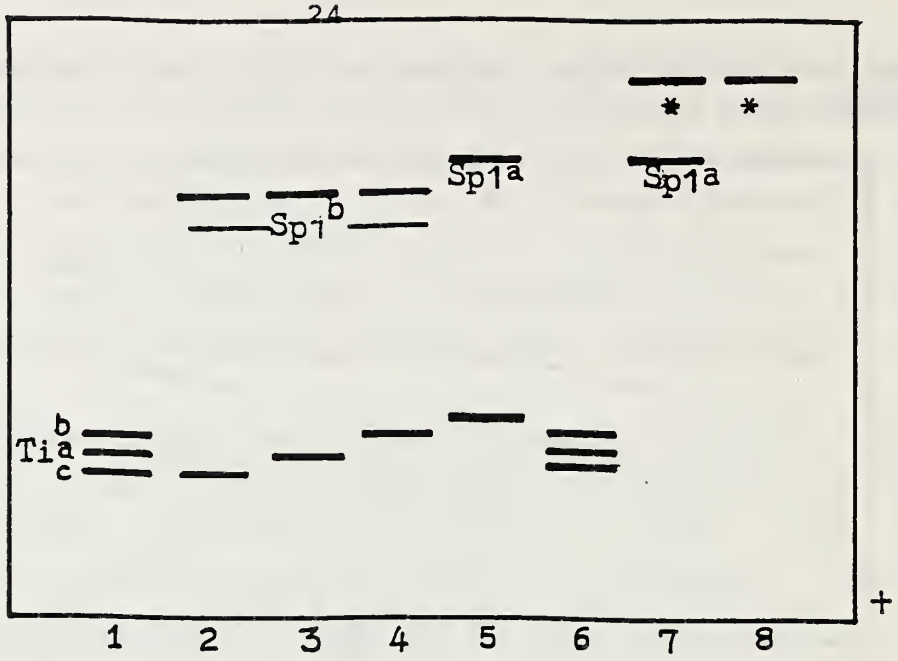


Figure 1

1, 6 - $\underline{Ti}^a$, $\underline{Ti}^b$, $\underline{Ti}^c$, markers; 2 - $\underline{Ti}^c$ genotype; 3 - $\underline{Ti}^a$ genotype; 4 - $\underline{Ti}^b$ genotype; 5 - new genotype; 7 - new type plus trypsin; 8 - Sigma Co. marker plus trypsin.

* - compound of SBTi and trypsin

References:
Hu Zhiang et al., 1983. Acta Bot. Sinica, 25(6):352-536.
Hymowitz, T. and N.Kaizuma. 1978. Soybean Genet. Newsl. 5:19-24.
Hymowitz, T. and H.H.Hadley, 1972. Crop Sci., 12:197-198.
Kunitz, T., 1945. Science, 10:668-669.
Orf, J.H. and T.Hymowitz. 1977. Crop Sci., 17:811-813.
Singh, L. et al. 1969. Crop Sci., 9:489-491.

Zhao Shuwen
Wang Hai

Soybean Research Institute
Nanjing Agricultural University
Nanjing, Jiangsu 210014
The People's Republic of China

1) Study on antixenosis and antibiosis of soybeans to the beanfly (Melanagromyza soja Zehntner)

The beanfly (Melanagromyza soja Zehntner) is one of the major pests in soybean production in southern China. It occurs extensively every year and is hard to effectively control by using insecticides. It is therefore suggested to use the resistance of soybeans.

Antixenosis: In order to study the adult ovipositional preference, four experiments (Expt. 1-4 in Table 1) with 12 to 27 soybean varieties were carried out in net rooms. At about flowering stage, the beanfly adults were artificially inoculated into the net rooms. Plants were sampled 48 hours after inoculation, and then stored in a refrigerator for inspection. The number of eggs per leaf was counted under microscope on about 1 cm in radius sector area of leaf, near the petiole where the beanfly adults usually oviposit their eggs. Results shown in Table 1 indicate that the average number of eggs per leaf of each variety was significantly different, suggesting that there existed a mechanism of antixenosis. Furthermore, adult beanflies tended to oviposit their eggs on the leaves of the middle and the upper part of a plant.

The next question was to reveal the mechanism of that kind of antixenosis. Two varieties, N3402 and N3562 with significantly different numbers of eggs per leaf, were chosen for an artificial inoculation experiment (Expt. 5). In a net room, 30 pots of N3402 (susceptible) plants were divided into six blocks (5 pots for each) and each block was treated with the following spraying treatments 55 days after planting: water, ethanol (95%), water extraction of N3562 (resistant), ethanol extraction of N3562 and control. In addition, in the same net room, six pots of N3562 plants were left untreated as control.

After all the treatments were done, adults of the beanfly were introduced into the net room. The number of eggs per leaf was counted two days later. Results showed (Table 2) that the leaves of N3402, when sprayed with water extraction and ethanol extraction from those of N3562, respectively, received significantly less eggs than those of N3402 did when sprayed with water and ethanol. It was inferred that there might be some specific chemicals in the water extraction and ethanol extraction of N3562, which might prevent adults from ovipositing their eggs. As to what the specific chemicals are, further study is needed.

Antibiosis: Four randomized block experiments with three replications were conducted under artificial inoculation in net rooms (Expt. 6 & 7) and natural infection in field (Expt. 8 & 9) to study the antibiotic manifestation of soybeans to beanfly and the interrelationship between the parasite and host.

In Expt. 6 and 7, 36 and 18 varieties were planted respectively in net rooms. The adults were inoculated just before flowering stage (r1~r2). Eight days after the inoculation, the plants were taken for inspection. The host traits included plant height, stem diameter, number of leaves per plant, number of pubescence in unit leaf area, leaf area, chlorophyll content, number of branches per plant, fresh leaf weight and dry leaf weight were examined first. Then the number of insects (larva and/or pupa) in stem (NIS), the number of insects in petiole (NIP), the number of insects in whole plant (NIW) were counted, respectively, by peeling the stem and petiole. Larvae and pupae from stem and petiole were weighed on a balance with accuracy of 1/1000000 g and measured by a vernier caliper at the accuracy of 1/100 mm.

Three resistant varieties and three susceptible varieties were used in Expt. 8 & 9, planted on June 13, 1989 in fields. On July 28, August 14, and September 14, the plants from the two experiments were peeled for NIS, NIP and NIW, and the pupae from the stem were weighed on a balance with accuracy of 1/1000000 g.

The results were as follows: 1) The weight of single larva in stem (WSLS), the length of single larva in stem (LSLS), and

the weight of single pupa in stem (WSPS) differed significantly among varieties (Table 3), indicating the presence of antibiosis of soybeans to the insect. However there has not existed a significant difference in the weight of single larva in petiole (WSLP) and the length of single larva in petiole (LSLP) among varieties. Thus, WSLS, LSLS, and WSPS might be major traits of antibiosis of the host. Furthermore, there existed significant difference on NIS, NIW (Table 3), and difference in the ratio of NIS to NIP.

2) The differences of NIW, NIP, and especially NIS among host genotypes became more and more obvious with delaying inspection (Table 4).

3) NIS was not significantly correlated with WSLS and LSLS, but there existed significantly positive correlation between the latter two traits, and also between the weight of single larva in whole plant (WSLW) and the length of single larva in whole plant (LSLW) (Table 5). These indicated that the antibiotic manifestation of number of insects was different from that of the weight or length of the insects, while that of the weight of the insects was basically the same, as was that of the length of the insects. In other words, resistance to boring of the insects from petiole to stem and resistance to growth and development of the insects in stem were two different antibiotic mechanisms. There existed germplasm with both mechanisms among the local races from southern China.

4) Under different environments, the number of insecticides in petioles, but not the number in petioles, tended to be relatively constant. The estimates of heritability of NIS, WSLS, LSLS were 57%, 41% and 40%, respectively. Therefore, NIS was chosen as the major indicator of resistance in breeding programs.

5) NIS and LSLS both were significantly correlated with stem diameter ($r=0.34^{\sim}0.38$), but not with plant height, number of leaves per plant, number of pubescence in unit leaf area, leaf area, chlorophyll content, number of branches per plant, fresh leaf weight, dry leaf weight.

Table 1. Analisys of variance for antixenosis experiments

Sources	Expt. 1 DF	Expt. 1 F	Expt. 2 DF	Expt. 2 F	Expt. 3 DF	Expt. 3 F	Sources	Expt. 4 DF	Expt. 4 F
Variety	15	3.58**	11	3.24**	18	4.45**	Variety	26	3.58**
Position	2	17.43**	2	35.85**	2	157.76**	Block	1	1.04
Var.×Pos.	30	1.22	22	1.36	36	1.23	Var.×Blk.	26	1.22
Error	190		144		224		Error	108	

** —— Significance at 1% level.

Table 2. Scott-Knott cluster analysis for the number of eggs per leaf of six treatments in Expt. 5

Treatment	Host	No. Eggs/leaf	Significance**
Water	N3402	2.22	A
Ethanol	N3402	1.41	B
Blank(S)	N3402	1.21	B
Water extraction*	N3402	0.69	C
Blank(R)	N3562	0.57	C
Ethanol extraction*	N3402	0.45	C

* — The leaves of N3562 were used for extraction.
** — The treatments with different letters A, B and C were significantly different at 1% lavel.

Table 3. F-test of variance analysis for Expt 6 to 9

Expt. 6
DF of variety=35
DF of error =140

NIS	WSLS	LSLS	NIP	WSLP	LSLP	NIW	WSLW	LSLW
7.55**	4.52**	4.37**	1.22	1.15	1.08	8.38**	5.33**	4.91**

Expt. 7 DF of variety=17 DF of error =34			Expt. 8 DF of variety=5 DF of error =10	Expt. 9 DF of variety=5 DF of error =10
NIS	NIP	NIW	WSPS	WSPS
6.56**	1.62	3.15**	6.44**	5.24**

** — Significant at 1% level.

Table 4. NIS, NIP and NIW (total from 40 plants) of six genotypes (Expt. 8 & 9)

Host	1st inspection (7/28)			2nd inspection (8/14)			3rd inspection (9/14)		
genotype	NIP	NIS	NIW	NIP	NIS	NIW	NIP	NIS	NIW
N1852	10.3	11.7	22.0	42.7	33.3	76.0	52.3	42.7	95.0
N2980	3.3	6.3	9.6	27.3	19.3	46.6	68.7	30.7	99.4
N3697	11.3	13.3	24.6	36.7	28.7	65.4	83.7	33.0	116.7
N2306	7.0	19.7	26.7	21.0	41.3	62.3	29.3	73.3	106.6
N2014	6.7	15.3	22.0	35.7	58.0	93.7	45.0	95.7	140.7
N10291	3.3	20.3	23.6	21.0	61.3	82.3	20.7	127.0	147.7

Table 5. Genotypic correlations among the larva traits (Expt. 6 & 7)

Traits	Range	NIS	WSLS	LSLS	NIW	WSLW
NIS	0.20~2.40					
WSLS	0.14~1.62	0.16				
LSLS	0.43~4.98	0.27	0.55			
NIW	1.24~4.66	0.91	0.30	0.25		
WSLW	0.35~1.55	0.15	0.65	0.50	0.07	
LSLW	1.04~4.18	-0.05	0.06	0.44	-0.17	0.42

References

Gai, J.Y., J.K. Xia, Z.L. Cui, Z.J. Ren, D.F. Ji. 1989. A study on resistance of soybeans from southern China to soybean agromyzid fly (Melanagromyza soja Zehntner). WSRC IV proceedings (3): 1240-1245. ASOJA, Buenos Aires, Argentina.

Gai Junyi, Xia Jikang, Cui Zhanglin, Ma Yuhua, Liu Fanyi, and Wei Tao. 1991. Inheritance of resistance to the beanfly (Melanagromyza soja Zehntner) in soybeans. Soybean Gene. Newsl. 18:104-106.

Wai T., Gai J., Xia J.K., Mar, II. 1989. Inheritance of resistance to beanfly (Melanagromyza soja Zehntner) in soybeans. Acta Gen. Sin. (China) 16(6):436-441.

Gai Junyi
Cui Zhanglin
Wei Tao
Liu Fanyi
Yan Huihuang

Soybean Research Institute
Nanjing Agricultural University
Nanjing, Jiangsu 210014
The People's Republic of China

1) Black root rot of soybeans in Jiangsu, China

Black root rot of soybeans caused by Cylindrocladium crotalariae (Loos) Bell & Sobers (teleomorph Calonectria crotalariae (Loos) Bell & Sobers) has been reported in Brazil, Cameroon, eastern Europe, Japan, Korea, and areas of the southeastern United States. It has become one of the major diseases of soybeans in some areas, but it has not been reported yet in China. This is a brief report of the finding of the disease in Jiangsu, China.

Investigation and observation: In 1987, some unknown diseased plants scattered in soybean fields were observed in Jiangpu Experiment Station of Nanjing Agricultural University. The most obvious symptoms included wilting, dying with or without defoliation, reddening of the stem near soil surface and root rot. It was once thought to be Sclerotium Blight (or White Mold).

Infected plants usually appeared to be scattered or confined to certain areas in infected soybean fields. Some patches of infected plants in seriously attacked fields could extend to as large as a hectare with almost no yield. The percentage of infected plants was estimated about 0.5%-20% in different fields during 1989 and 1990. The disease could attack both summer-planting soybeans and spring-planting soybeans. Wilting usually appeared in the early pod stage (R3-R4) and dying before maturity. It seemed that there existed differences of resistance among varieties. Soybean cultivar Nan-Nong 73-935 and others seemed to be resistant with a low percentage of infected plants, while cultivar 1138-2 and Nan-Nong 86-4 seemed to be susceptible with a high percentage of infected plants. The occurrence of the disease appeared to be related to rotation and cropping systems. Disease was serious in the fields with a long-time wheat-soybean

rotation and negligible in the fields with wheat-rice-wheat-soybean rotation. The disease was less serious under rotation with cotton than under continuous soybeans.

For a detailed description, 60 plants were labelled for observation of development of the disease in a summer-planting soybean field, seriously attacked for several years, in 1990. On August 15, a few white velvet hyphae were found on the stems near soil surface. Some hyphae proliferated onto soil surface around the plants. On the upper part of the plants there was no symptom of the disease. After August 20, the stems near soil surface became reddish-brown with or without ulcer, interveinal tissues of top leaves turned light brown, and the leaves began temporary wilting during daytime. On August 29, all the 60 plants appeared wilting and were unable to recover. Necrosis of leaves occurred afterwards. Tiny reddish-orange perithecia were developed on infected stem tissue near soil surface which were sometimes reddish even in the absence of the perithecia. The perithecia were also found on roots under soil surface. At this time, the roots were black and rotted, and tissues within the stalk became grayish-brown. All 60 labelled plants died before September 15.

Isolation and inoculation: In order to find the cause of the disease, infected plants were brought to laboratory for examination and isolation in 1988. Under the microscope, the cylindrically straight hyaline and three-septate conidia, and reddish-orange and globe-like perithecia which contained clavate asci-bearing curved hyaline mostly three-septate ascospores of Calonectria crotalariae were observed on 76 infected plants out of 80 plants sampled from 6 places in Jiangpu and Nanjing.

A number of diseased plants with wilting and root rot were sampled in 1989 and 1990 for isolation. Pieces of root and stalk tissues were surface-sterilized before plating in potato-dextrose agar in Petri dishes. Calonectria crotalariae was isolated from 19 dishes out of 30 dishes in 1989 and nearly all the dishes out of 40 dishes in 1990.

To detect the fungus associated with the disease, Koch's postulates were fulfilled by growing plants in the artificial

climate chamber, greenhouse and field where the soil was artificially infected with Calonectria crotalariae. The results showed that symptoms appeared on the inoculated plants were the same as appeared on the naturally-infected plants in the field and Calonectria crotalariae as again isolated from the inoculated plants.

Comparing the symptoms and the causal fungus of the disease with those of others, it was quite evident that the disease observed in Jiangpu Experiment Station was Black Root Rot of soybeans, not Sclerotium Blight of soybeans.

After further investigation, Black Root Rot of soybeans was also found in Nanjing, Yangzhou, Yangcheng, Huaiyin, Guanyun, Taixing, Jinjiang, Nantong etc. Calonectria crotalariae was observed on the diseased plants from all the places above.

References

Bell, D.K. and E.K. Sobers. 1966. A peg, pod, and root necrosis of peanut caused by a species of Calonectria. Phytopathology 56:1361-1364.

Berner, D.K., G.T.Berggren, M.E.Pace, E.P.White, J.S.Gerahey, J.A.Freedman and J.P.Snow, 1986. Red crown rot: Now a major disease of soybean. La.Aric. 29:4-5, 24.

Roy, K.W., K.S.McLean, M.V.Patel, and W.F.More, 1989. First report of red crown rot on soybeans in Mississippi. Plant Dis. 73:273.

Sinclair, J.B. (ed.), 1975 (1st edition), 1982 (2nd edition), 1989 (3rd edition). Compendium of Soybean Diseases. American Phytopathological Society. 3340 Pilot Knob Road, St. Paul, MN.

Gai Junyi
Cui Zhanglin
Lin Maosong

JILIN ACADEMY OF AGRICULTURAL SCIENCES
Soybean Research Institute
Gongzhuling, Jilin Province 136100
The People's Republic of China

1) A preliminary study on genetic variation of major yield characters of progeny from G.max X G.gracilis hybrid*

Progeny from three crosses of G.max X G.gracilis were used to study the effect of different terminal types on yield characters of progeny.

Materials and methods: Three crosses among three cultivated soybean with different terminal types and a semi-wild soybean (G.gracilis) gene types were made. Performance of parents can be seen in Table 1.

Table 1. Performance of characters of parents

Characters	Tongnong 9 G.max	Jilin 16 G.max	Gongjiao 7515 G.max	GD50856 G.gracilis
Stem determination	Det.	Ind.	Semi-det.	Ind.
Growth type	Erect	Erect	Erect	Vining Growth
# Pods per plant	79.6	83.2	83.4	351.2
# of seeds per plant	190.4	188.2	204.2	807.2
Weight of 100 seeds (g)	17.1	17.2	18.8	4.1
Seed yield per plant	31.5	32.1	37.8	31.8

Cross 1: Tongnong 9 * GD50856
Cross 2: Gongjiao 7515 * GD50856
Cross 3: Jilin 16 * GD50856

The parents, F1 and F2 generations of each cross were grown in 1988 at Gongzhuling, Jilin.

*This project is supported by NNSF of China

The characters studied were: number of pods per plant, number of seed per plant, weight of 100 seeds and seed yield per plant.

Results and discussion: Data in Table 2 showed that, except the weight of 100 seeds, heterosis was found for all other characters in F1 generation. Heterosis indices were the highest in seed yield per plant (146.45). Heterosis indices for all characters decreased in the F2 generation, in general, Cross 1 > Cross 3 > Cross 2.

Table 2. Heterosis indices of yield characters in F1 and F2

Characters	Cross 1		Cross 2		Cross 3		Means	
	F1	F2	F1	F2	F1	F2	F1	F2
No. pods per plant	134.5	121.3	94.2	91.9	101.9	99.8	110.2	104.3
No. seeds per plant	162.1	121.2	95.7	92.0	102.0	98.5	119.9	103.9
Weight of 100 seeds	87.7	85.9	83.8	78.6	82.6	81.7	84.7	82.1
Seed yield per plant	179.0	167.5	124.5	123.0	135.8	130.8	146.5	140.1

The GCV of seed weight per plant Cross 1 > Cross 2 > Cross 3 >. Except for 100-seed weight, some transgressive segregates over larger parents were found for all other characters in the F2 generation. Seed weight per plant was larger than that of the bigger parent from all crosses tested. Plants of 110 cm in height, 350 pods per plant and erect main stem were separated from Cross 1 and Cross 2.

Data in Table 4 showed that high heritability was found in 100-seed weight: therefore, selection would be effective in early generation. Low heritabilities were found in all other characters and selections based on such characters should be performed in later generations. Larger expected genetic advances absolute value were found in all characters. In general, Cross 1 > Cross 2 > Cross 3.

Table 3. The means, Ranges, and GCV.s of yield characters in F2

Characters	Cross	Means	Ranges	GCV.		
				%	Means	Place
No. pods per plant	1	261.3a*	99-497	26.73	27.09	2
	2	178.0b	53-383	36.53		
	3	216.8b	131-425	17.01		
No. Seeds per plant	1	604.4a	281-1180	19.38	25.07	3
	2	364.2c	107-651	36.4		
	3	491.5b	262-872	19.44		
Weight of 100 seeds	1	9.1a	7.3-13.3	21.12	13.4	4
	2	9.3	6.5-10.5	10.58		
	3	8.7a	7.8-10.7	8.49		
Seed yield per plant	1	58.3a	21.9-99.1	55.42	38.18	1
	2	38.6b	15.9-48.6	36.18		
	3	41.8b	26.4-62.4	22.95		

* The means within a character followed by a same letter are not significantly different between crosses at the 0.05 probability.

Table 4. The heritabilities and expected genetic advances of yield characters in F2

		Heritability			Expected genetic advances	
Characters	Crosses	%	X	Place	5% select rate	5% absolute value
No. of pods per plant	1	17.33	17.66	4	23.78	30.16
	2	13.58			27.73	23.96
	3	22.08			16.46	14.71
# of seeds per plant	1	44.57	30.23	3	26.65	78.20
	2	30.51			41.42	73.23
	3	15.62			15.83	37.76
Weight of 100 seeds	1	57.39	59.73	1	32.96	1.46
	2	50.83			15.54	0.70
	3	70.98			14.73	0.62
Seed yield per plant	1	44.43	38.48	2	76.10	21.51
	2	37.10			45.40	8.51
	3	33.92			27.53	5.59

The results showed that in the crosses between cultivated soybean (G.max) with determinate growth habit and semi-wild soybean (G.gracilis) plants with good yield characters can be separated. It is possible to improve yield through the interspecific cross between G.max and G.gracilis.

References

Wang Jinling et al. 1986. Effect of backcrossing on overcoming viny and lodging habit of cultivated X wild and cultivated X semi-wild cross. Soybean Sci. 3:181-188.

Gai Junyi et al. 1982. Genetic performance of several agronomic characters in four generations of back crossing program between cultivated and wild soybean. Acta Genetica Sinica 9:44-56.

Yang Guangyu et al. 1990. A study on the use of G.soja to breed new soybean cultivars. Sci. Agri. Sinica 1:89-90.

Li Wenbin et al. 1987. A comparative study of inheritance of several agronomic characters among crosses between G.soja, G.gracilis and G.max. Soybean Sci. 4:265-275.

A.R. LeRoy et al. 1991. Introgression of genes for small seed size from Glycine soja into G.max. Crop Sci. 31:693-697.

Yang Guangyu
Zheng Huiyu
Han Chuenfen

3) Frequency and geographical distribution of Ti and Sp_1 Alleles in seed storage protein of semiwild soybean in China*

Semiwild soybean (G.gracilis) is a transitive type from wild soybean (G.soja) to cultivated soybeans (G.max). The frequency of Ti (SBTi-A2) and Sp_1 allele in seed storage protein of semi-wild soybean was analyzed in this experiment.

Materials and methods: Samples used in this experiment were grown and stored by our laboratory. One hundred and twenty-six samples were collected from a northern region (40°-53°N), 76 samples were collected from a central region (30°-40°N), and 44 samples were collected from a southern region (24°-30°N) of China. The weight of one hundred seeds was from 2.5 to 10 grams.

Extraction of Ti followed the method modified by Hymowitz et al. (1972), and gel electrophoresis followed the method modified by Hu et al. (1983). Markers were from Sigma Co.

Results and discussion: The results in Table 1 showed that there were only Ti^a and Ti^b alleles in Ti locus, and Sp_1^a, Sp_1^b and null (sp_1) allele in Sp_1 locus in semiwild soybean. The frequency of Ti^a and Sp_1^a in a central region was higher than that in the northern and southern regions. These results coincide with the results we obtained from wild soybean in China.

The frequency of Ti^a in 4492 cultivated soybeans from central region we examined was 99.5%. It can be seen that the frequency of Ti^a increased as the soybean evolved from wild to semi-wild and cultivated soybean. The frequency of Ti^a was closest in the central region of China. This result is valuable for studies on origin and evolution of soybean.

Table 1. Frequency and geographical distribution of Ti and Sp_1 in semiwild soybean in China

		Ti			
°N	# samples	Ti[a]	%	Ti[b]	%
40°1'-53°	126	114	90.5	12	9.5
30°1'-40°	76	74	97.4	2	2.6
24-30°	44	35	79.5	9	20.5
total	246	223	90.7	23	9.3

		Sp_1					
°N	No. of samples	Sp_1[a]	&	Sp_1[b]	%	Sp_1	%
40°1'-53°	126	16	12.7	102	81.0	8	6.3
30°1'-40°	76	17	22.4	59	77.6	0	0
24-30°	44	5	11.3	38	86.4	1	2.3
total	246	38	15.4	199	80.9	9	3.7

Table 2. Frequency and geographical distribution of Ti and Sp_1 allele in wild soybean in China

		Ti					
°N	# of samples	Ti[a]	%	Ti[b]	%	Ti[c]	%
40°1'-53°	82	60	73.2	22	26.8	0	0
30°1'-40°	171	164	95.9	6	3.5	1	0.6
24-30°	86	50	58.1	36	41.9	0	0
total	339	274	80.8	64	18.9	1	0.3

Sp_1

°N	No.of samples	$Sp_1{}^a$	%	$Sp_1{}^b$	%	Sp_1	%
40°1'-53	82	17	20.7	64	78.0	1	1.2
30°1'-40°	171	104	60.8	67	39.2	0	0
24-30°	86	40	46.5	46	53.5	0	0
total	339	161	47.5	177	52.2	1	0.3

Zhao Shuwen
Zhou Shuhua
Xu Bao

GUANGDONG PROVINCIAL ACADEMY OF AGRICULTURAL SCIENCES
Agricultural Biotechnology Research Institute
Wushan, Guangzhou 510640, China
and
CHINESE ACADEMY OF AGRICULTURAL SCIENCES
Institute of Crop Germplasm Resources
Baishiqiao, Beijing 100081, China

Behavior of different cold-tolerant genotypes of soybean

A series of our studies on soybean for tolerance to cold have been published since 1989 (Li Yujun et al., 1989, 1990, and 1991). We haven't given more details about the behavior of different genotypes of soybean for tolerance to cold until the following report, which show behavior of germination, emergence, days to maturity, plant characteristics, yield, and changes of physiology, etc., of different cold-tolerant genotypes of soybean.

Materials and methods: After screening of soybean genotypes for cold tolerance during germination, six varieties (in which there were three for tolerance to cold and three for intolerance) were tested as follows:

Germination tests - Dry seeds were pretreated and then germinated in low temperature and ambient temperature according to our preceding thesis (Li Yujun et al., 1989).

A paired field trial - Soybean seeds treated in low temperature of 6° for 14 days similar to the preceding studies (Li Yujun et al., 1989) were sowed in the field while dry seeds that weren't given any treatment were planted randomly in pairs. Days to emergence, days to flowering, days to maturity, plant characters, and yield were measured in the field.

Laboratory tests - Soybean seeds reaped from the field tests were taken to do a series of physiological experiments. Several characters of physiology, such as rate of water uptake, rate of leakage, and superoxide dismutase (SOD), etc., were measured in the laboratory according to our preceding reports (Li Yujun et al., 1991).

Results and discussion: Classification of cultivars into cold-tolerant and intolerant groupings - After a number of soybean cultivars were germinated at 6°C and 25°C, the relative germination ratios (RGR) were calculated according to a formula (Li Yujun et al., 1989). Then three ranks of tolerance to cold were graded with the formula. Two genotypes of soybean were graded for cold tolerance (one cold-tolerant, the other intolerant) (Table 1), were selected for the following experiments. In the germination tests, ZDD6965, ZDD7149 and ZDD7201 were classified as cold-tolerant genotypes, due to their higher RGR; the others were cold-intolerant.

Table 1 - Relative germination ratios of soybean

Varieties	ZDD 6965	ZDD 7127	ZDD 7149	ZDD 7194	ZDD 7201	ZDD 0656
RGR %	95.1	26.0	92.5	19.2	80.0	29.7

Emergence - Although the emergence potentials or the final emergence percentages of cultivars sowed directly in the field were not significantly different among them, those of same seeds treated in low temperature of 6°C for 14 preceding days, then planted in the field, differed greatly and were divided into two groups. In the field tests, two of six cultivars, ZDD7149 and ZDD7201, were cold-tolerant, and the others were intolerant or intermediate, due to their lower ability to emerge (Table 2). The same trends between emergence potential and final emergence percentage were shown in the paired experimental design.

Days to emergence, days to flowering and days to maturity: Days to emergence for all cultivars treated in low temperature in advance and then sowed in the field were 2-3 days longer than those sowed directly in the field, as well as days to flowering and days to maturity in our observations, which may transfer more photosynthate to produce seed yield.

Table 2 - Behaviors of soybean genotypes for cold tolerance

Varieties	ZDD 6965	ZDD 7127	ZDD 7149	ZDD 7194	ZDD 7201	ZDD 0656
Emergence potential (%)	63.3 12.7	63.3 16.7	76.0 31.3	72.0 0.0	84.0 46.7	39.3 2.7
Final emergence %	84.7 36.7	83.3 42.7	93.3 56.0	87.3 4.7	96.7 68.0	72.7 6.0
Plant height cm	62.7 53.7	71.7 70.3	55.1 50.8	45.8 22.9	54.8 55.5	69.4 77.9
No. of branches	1.1 0.8	2.1 2.3	3.5 6.3	2.1 6.2	2.3 3.0	3.7 4.3
No. of pods	39.1 47.6	44.2 66.2	63.4 85.6	45.3 55.1	41.5 57.4	94.7 136.2
Yield per plant (g)	16.2 19.9	19.7 30.1	21.6 28.0	19.6 23.6	14.0 20.1	29.8 37.8
Yield per plot (g)	381.7 158.3	591.7 261.7	590.0 411.7	508.3 45.9	473.3 353.3	588.3 104.5
Water content %	7.51 7.57	7.83 8.37	6.65 7.15	6.96 7.63	7.58 7.49	7.71 8.16
Water uptake mg/seed	133.8 141.0	133.4 136.7	117.8 121.9	138.2 140.5	104.7 80.4	128.9 106.7
Rate of water uptake mg/seed*h			2.54 1.81	3.90 2.78	2.37 1.88	2.94 2.37
Electrical conduc-tivity	0.86 0.89	0.78 0.59	0.49 0.34	0.74 0.56	0.25 0.16	0.60 0.41
Rate of EC(μv/cm*seed*h)			0.16 0.12	0.32 0.19	0.15 0.11	0.22 0.17

*Above numbers represent control, bottom numbers represent treated soybean

Characters of plant and yield: Plant heights of soybean seeds treated in low temperature in advance and then sowed in the field were seldom increased, when compared with those sowed

treated soybean were quite different from those of control. The former always increased markedly (Table 2), which may have more chance and ability to make seed yield. The yield per plant of soybean treated at low temperature during germination was significantly higher than that of soybean planted in the field directly (Table 2), suggesting a correct inference above. Because of seed damage of cold pretreatment, soybean seed yield per plot decreased greatly, except ZDD7149 and ZDD7201. Cold-tolerant genotypes (Table 2) were proved by the tests of germination and emergence etc. above.

Physiology: Water content of soybean seeds reaped from the field, which were pretreated in the low temperature before sowing, was almost always higher than that of control, with the opposite of electrical conductivity. The water uptake of soybean seeds had little variation between treatment and control. It is clear that soybean seeds that were pretreated in low temperature during germination before sowing had an ability to resist cold stress.

There were very interesting results in the polyacrylamide gel electrophoresis of SOD extracts of soybean seeds or hypocotyls, and the screening of electrophoretic SOD bands for scanning. The enzyme bands of SOD in cold-susceptible genotypes were quite different from pretreated seeds and control seeds. The peak value of SOD band in treated soybean was higher than that in control (Fig. 1). This certifies the ability to resist more strongly to cold stress in treated seed especially for intolerant genotypes, because SOD is one of the enzymes that eliminates the ions such as O, HO, OH etc. Thus tolerant and intolerant soybean pretreated in cold conditioning water during germination before sowing was improved in cold tolerance.

References:

Li Yujun, Chang Ruzhen, and Zhao Yutian. 1989. Response of soybean to low temperature during germination. Soybean Genet. Newsl. 16:33-37.

___, Chang Ruzhen, Zhao Yutian, and Sun Jiangyin. 1990. Application studies in the field on screening for cold-tolerance in the laboratory in soybeans. Soybean Genet. Newsl. 17:32-42.

References:
Li Yujun, Chang Ruzhen, and Zhao Yutian. 1989. Response of soybean to low temperature during germination. Soybean Genet. Newsl. 16:33-37.
___, Chang Ruzhen, Zhao Yutian, and Sun Jiangyin. 1990. Application studies in the field on screening for cold-tolerance in the laboratory in soybeans. Soybean Genet. Newsl. 17:32-42.
___, Chang Ruzhen, and Zhao Yutian, 1991. Inbibitional chilling injury in cultivars of soybean. Soybean Genet. Newsl. 18:68-73.
___, Zhao Yutian, and Chang Ruzhen, 1991. The effect of polyethylene glycol and ascorbic acid on soybeans for cold tolerance. Soybean Genet. Newsl. 18:73-77.

Li Yujun, who is now with Agricultural Biotechnology Research Institute of GAAS.
Chang Ruzhen
Zhao Yutian
Sun Jiangyin

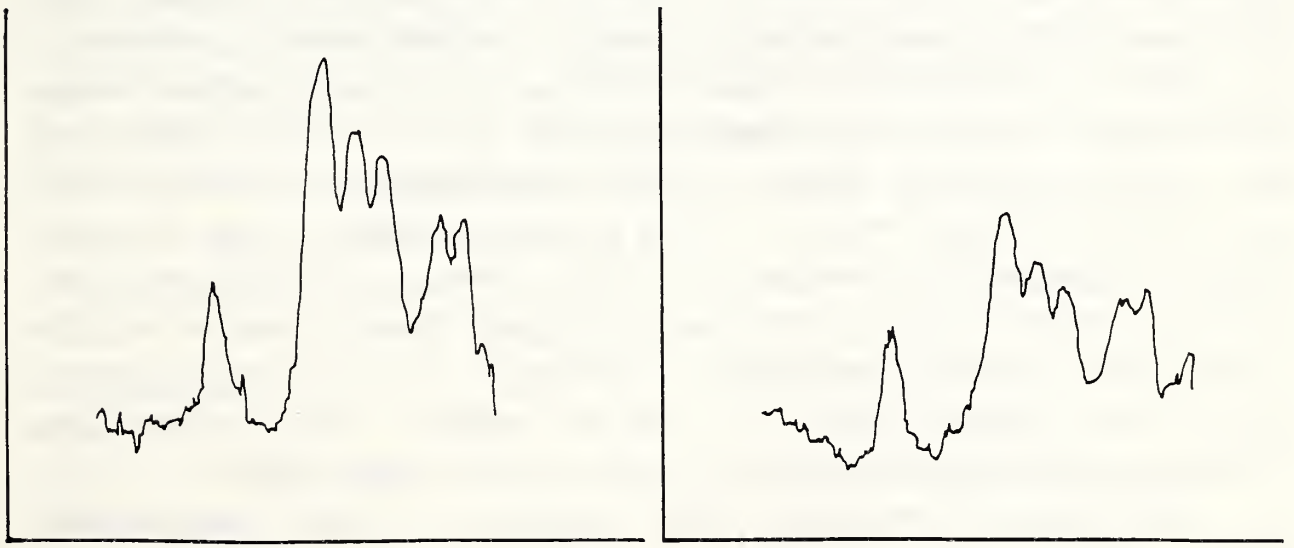


Fig. 1 Scanning of electrophoretic SOD bands of seed hypocotyls of ZDD0656 during germination at 6 C.

Northeast Agricultural College
CHINA

Impact of plant growth regulators and pH on somatic embryogenesis and efficient plant regeneration in Glycine tomentella (Hayata) originated from South China

Introduction: Perennial soybeans of the subgenus Glycine have been much emphasized to be exploited in soybean breeding program, since they possess agronomically valuable characteristic, involving pesticide and disease resistance, drought and cold tolerance, apparent daylength insensitivity. Many attempts have been made to introgress them into cultivated soybean by conventional breeding techniques in recent years (Broue et al., 1982; Newell et al., 1982; Brown et al., 1985; Singh et al., 1985; Newell et al., 1986; Singh et al., 1987). However, those efforts have been less successful because of extremely low crossability and early pod abortion. Along with the development of biotechnology, it seems to be more hopeful to overcome the barriers by means of somatic hybridization. Hereupon, plant regeneration system via embryogenesis or organogenesis should be established in advance.

Plant regeneration from perennial relatives of soybean have been successful in G.canescens (Widholm et al., 1983; Hammatt et al., 1987, 1989; M.R. Davey., 1987); G.clandestina (T.Hymowitz et al., 1986; Krishna et al., 1989); G.latrobeana (B.Jon et al., 1988); G.argyrea (B.Jones et al., 1989), mostly via organogenesis. In our present report we described efficient plant regeneration via embryogenesis is G.tomentella originating in South China and proved that plant growth regulators and pH were two key factors for effective embryogenesis and successful plant regeneration.

Materials and methods: Wild perennial species G.tomentella PWS-6, which was identified as having partial tolerance to glyphosate (unpublished), was planted at experiment station of NEAC n 1990 and immature pods containing seeds 0.5-4.5mm long were harvested, following a storage at 4°C. Then surface sterilized pods by immersing for 30 sec. in 70% ethanol followed by 10 min.

embryogenesis

According to previous reports in the subgenus soja (T.Komatsuda, Wenbin Lee, et al., 1991), the competent somatic embryogenesis occurred on cotyledons from immature seeds 1/2 size of maturity stage in length. In contrast, present results indicated that cotyledons from earlier stages of seeds produced the highest numbers of somatic embryos in G.tomentella.

MSB medium containing MS salts and B5 vitamins was popularly adapted to soybean embryogenesis procedure. This medium also was a critical factor in our experiment and attention was focused on the response of auxin and pH value to somatic embryogenesis of G.tomentella.

Of three concentration levels of NAA, no embryogenesis was achieved on medium EI with 1mg L^{-1} NAA except of high proportion of induction of green calli, and cotyledons on medium EI with 5mg L^{-1} NAA displayed high capacity of embryogenesis, the frequency of embryogenesis reaching 60.2% and efficiency of embryogenesis becoming 1.71. As a feature of effective embryogenesis, most of calli manifested a black-brown color (Table 1). However, generation and germination of somatic embryo was significantly hindered under higher content of NAA (10mg L^{-1}); meanwhile, rate of black-brown calli was diminished to 17.25%. Many reports indicated that higher levels of auxin were necessary for vigorous embryogenesis in the subgenus soja (Paul A. Lazzeri et al., 1986), but, to date, less information was available for the species of the subgenus glycine. Present result clearly demonstrated that somatic embryogenesis of G.tomentella could be prompted by adding less NAA to medium EI, especially 5mg L^{-1}, instead of 10mg L^{-1} which was beneficial to Soja species. The difference possibly was due to more supplement of inner auxin or a limited requirement of auxin to cell differentiation in G.tomentella

immersion in 2.5% commercial hypochlorite solution with one drop of triton X-100. Embryos were excised from seeds and divided into two cotyledonary explants. Explants were then inoculated in tubes with 10 ml of gelrite-solidified medium EI composed by MS salts (Murashige and Skoog 1962), B5 vitamins (Gamborg et al., 1968), 1% sucrose, 1-10mg L^{-1} naphthaleneacetic Acid (NAA), 0.22% gelrite, with pH 5.0-8.0.

Maturation culture of somatic embryos was conducted after 4 weeks. Somatic embryos were subcultured every two weeks for four weeks.

After maturation, somatic embryos were moved onto germination medium EG containing 1/2 MS salts and B5 vitamins plus 0.001-0.1mg L^{-1} Gibberellin (mainly GA3), 1% sucrose and subcultured every week for three weeks. Completely randomized design was adopted in this experiment with two replications.

Results and discussion: To evaluate reaction of auxin and pH to embryogenesis in G.tomentella, previously we screened the optimal seed stage that was capable of inducing somatic embryos. Figure 1 showed that earlier stage of seed possessed the highest competence for embryogenesis in G.tomentella. Seed length 1±0.5mm was the best size, as a seed elongated, frequency of embryogenesis decreased greatly; embryogenesis from seeds that were near to physiological maturation (seed 4±0.5 mm long) was inhibited.

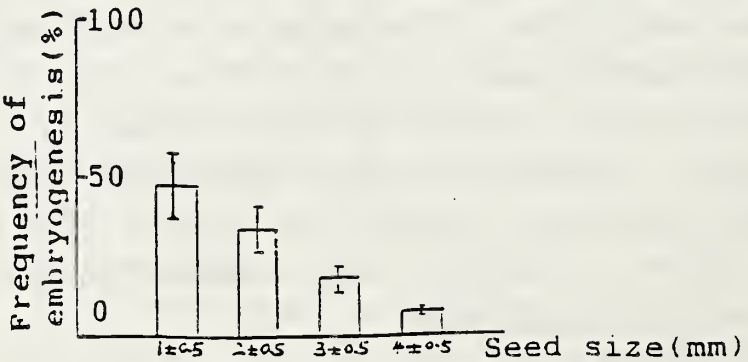


Fig. 1 - Relationship between seed size and frequency of embryogenesis

Table 1 - Effect of NAA concentration on embryogenesis in G.tomentella

Indexes / NAA (mg L^{-1})	Green Calli %	Brown Calli %	Frequency of Embryogenesis %	Efficiency of Embryogenesis
1	64.61	4	0	0
5	31.67	61.16	60.20	1.71
10	57.25	17.25	9.45	0.35

Seven pH treatments were offered to appraise the effect on callus formation and cell differentiation. As a result (Table 2), under pH 5.0, no tissue growth was observed due to a heavy toxicity from ionic action, while individual calli occurred under pH 5.5 and pH 8.0, suggesting that immature embryos isolated from G.tomentella seeds possessed a preferential tolerance to acidity and basicity. Somatic embryos were induced in medium EI with pH range from 6.0-7.5 and the optimal pH value was 7.0 in which frequency of embryogenesis rose to 51.14%, efficiency of embryogenesis was increased to 2.33.

Table 2 - Effect of pH value on embryogenesis in G.tomentella

Indexes / PH Value	Green Calli %	Brown Calli %	Frequency of Embryogenesis %	Efficiency of Embryogenesis
5.0	5	0	0	0
5.5	28.22	0	0	0
6.0	9.22	60.17	8.94	0.67
6.5	16.61	79.17	23.26	0.90
7.0	13.45	86.56	51.14	2.33
7.5	4.22	61.22	17.09	0.72
8.0	8.17	8.45	0	0

Reviewing previous work, pH value in media was mostly adjusted to 5.8 prior to autoclaving in soybean organogenesis and somatic embryogenesis could be derived from immature embryos of cultivated soybean incubated on medium EI with pH 7.0 (T.Komatsuda, Wenbin Lee, et al., 1991). Recently a similar phenomenon was revealed from G.tomentella.

In addition, data analysis demonstrated there was an apparent relationship between callus characteristics and embryogenesis in G.tomentella. Black-brown calli more effectively qualified for generation of somatic embryos (R=0.79) and could be made as a marker of desired embryogenesis.

Alike the species in subgenus Soja, there also had maturation and germination procedure for G.tomentella somatic embryos. During maturation culture, the color of embryos was gradually altered from green to yellow. Moreover, maturated somatic embryos rarely germinated without the addition of gibberellin to medium EG. Table 3 a mini-amount of gibberellin (0.001mg L^{-1}) was available for root formation, but not sufficient for shoot formation, and effective generation of plantlet occurred with the existence of 0.05mg L^{-1} gibberellin in medium EG.

Table 3 - Effect of gibberellin on germination of somatic embryos in G.tomentella

Indexes / Auxin Level (mg L^{-1})	With Root %	With shoot %	Both With Root and Shoot %
0	11.17	9.19	8.52
0.001	44.06	44.37	25.83
0.005	42.03	47.79	28.61
0.01	43.01	52.41	30.50
0.05	48.40	62.61	46.11
0.1	48.59	55.94	35.00

References:

Broue P. et al. 1982. Euphytica 31:715-724.
Brown A.H.D. et al. 1985. In: Proc. World Soybean Research Conf. R.Shibles, (ed) pp.345-352. Westview Press, Boulder, CO.
Hammatt N. et al. 1987. Plant Sci. 48:129-135.
___, et al. 1989. Soybean Genet. Newsl. 16:65-67.
Jones B. et al. 1988. Soybean Genet. Newsl. 15:51-53.
Krishna P. Kollipara et al. 1989. Soybean Genet. Newsl. 16:83-84.
M.R.Davey et al. 1987. Soybean Genet. Newsl. 14:45-47.
Newell C.A. et al. 1982. Crop Sci. 22:1062-1065.
___, et al. 1986. Conference on Molecular and Cellular Biology of the Soybean, pp.5, Iowa State University, Ames, IA.
Paul A. Lazzeri et al. 1986. Planta 167:473-481.
Singh R.J. et al. 1985. Euphytica 34:187-192.
___, et al. 1987. Theor. Appl. Genet. 74:391-396.
T.Hymowitz et al. 1986. Plant Cell Reports. 3:192-196.
T.Komatsuda, Wenbin Lee, et al. 1991. Plant Cell. Tissue Organ Culture 26.
Widholm J.M. et al. 1983. Plant Cell Reports 2:19-20.

Wenbin Lee

INDIA

GOVIND BLLABH PANT KRISHI EVAM PRAUDYOGIK
Vishwa Vidalaya, Pantnagar, UP-263145 India

1) Yield components in determinate vs. indeterminate soybeans

Soybean cultivars are described as having determinate ($\underline{dt}_1$), semi-determinate ($\underline{dt}_2$) or indeterminate ($\underline{Dt}_1$) stem termination (Bernard, 1972). In India, determinate cultivars predominate in the northern hills and plains while semi-determinate and indeterminate ones are largely grown in the central and the southern parts of the country. However, not much work has been done in this tropical situation to identify seed yield components/combination of traits having a direct bearing on seed yield improvement, separately in both the sets (determinate vs. indeterminate) of varieties. Therefore, this experiment involving 10 determinate and 10 semi-indeterminate soybean cultivars was undertaken.

Materials and methods: Twenty varieties (10 determinate and 10 semi-indeterminate = indeterminate) were evaluated in a randomized block design with four replications during rainy season of 1989. Each plot consisted of three rows which were 4 m long, spaced 60 cm apart. Plant-to-plant distance was maintained at about 15 cm. Observations recorded (Table 1) were subjected to correlation analysis (Fisher, 1954).

Results and discussion: Based on significant phenotypic correlations of agronomic traits with seed yield and supported by substantially high genotypic correlations (Table 1), several characters (days to flowering, days to maturity, plant height, branches on the main stem, total branch nodes on the main stem, nodes on primary branches, pods on branches, total pods, and lodging) were shown to be important characters influencing seed yield positively in indeterminate varieties. Generally, these characters have been found to be important yield components by various authors (Weber and Moorthy, 1952; Anand and Torrie, 1963; Byth et al., 1969; Wilcox, 1980; Bramal et al., 1984; and Lin and

Nelson, 1988).

For determinate varieties the results were sharply different where plant height and pods on main stem, pod on branches and total pods turned out to be the major yield components. Number of pods on the main stem was not at all important in indeterminate varieties. In support of these results, roles of plant height in increasing seed yield has been shown by Ablett et al. (1989) who reported that yield of the determinate stem type soybeans was generally positively associated with plant height and maturity and suggested that the stringent use of these as selection criteria in early generations may provide a relatively simple way of increasing the frequency of favorable determinate genotypes.

Conclusion: The evaluation of ten determinate and ten semi-determinate/indeterminate soybeans indicated that several characters (days to flowering, days to maturity, plant height, branches on main stem, total branches, nodes on main stem, nodes on primary branches, pods on branches and total pods) proved to be the major yield components for indeterminate soybeans. In contrast, only plant height, pods on main stem, pods on branches and total pods were the important yield components for determinate soybeans.

References

Ablett, G.R., Beversdorf, W.D. and Dirks, A. 1989. Performance and stability of indeterminate and determinate soybean in short-season environments. Crop Sci. 29:1428-1433.

Anand, S.C. and Torrie, J.H. 1963. Heritability of yield and other traits and the interrelationships among traits in the F_3 and F_4 generations of three crosses. Crop Sci. 3:508-511.

Bernard, R.L. 1972. Two genes affecting stem termination in soybeans. Crop Sci. 12:235-239.

Bramel, P.J., Hinz, P.N., Green, D.F. and Shibles, R.M. 1984. Use of principal factor analysis in the study of three stem termination types of soybean. Euphytica 33:387-400.

Byth, D.E., Caldwell, B.E. and Weber, C.R. 1969. Correlated truncation selection for yield in soybeans. Crop Sci. 9:699-702.

Fisher, R.A. 1954. Statistical methods for research workers. 12th ed. Oliver and Boyd Ltd., London.

Lin, M.S. and Nelson, R.L. 1988. Effect of plant height and flowering date oMwseed yield of determinate soybean. Crop Science 28:218-222.

Weber, C.R. and Moorthy, B.R. 1952. Heritable and non-heritable relationships and variability of oil content and agronomic characters in the F_2 generation of soybean crosses. Agron. J. 44:202-209.

P.K. Bhattacharyya
Hari Har Ram

Table 1. Correlations between character pairs involving only seed yield in case of determinate varieties and indeterminate varieties.

	Determinate			Indeterminate		
Character pairs involve seed yield	r_g	r_p	r_e	r_g	r_p	r_g
Days to flowering vs. yield	0.451	0.256	-0.345*	0.764	0.688*	0.074
Maturity vs. yield	0.425	-0.347*	0.079	0.770	0.680**	-0.008
First to last flowering on main stem vs yield	-0.309	-0.264	-0.157	0.261	0.251	0.203
First to last flowering on branches vs. yield	-0.244	-0.155	0.043	0.393	0.352*	0.120
Plant height vs. yield	0.758	0.635**	0.121	0.804	0.724	0.201
Branches on main stem vs. yield	0.207	0.244	0.340	0.922	0.492	0.151
Total branches vs. yield	-0.045	0.024	0.096	0.881	0.723**	0.416**
Nodes on main stem vs. yield	-0.083	-0.115	-0.190	0.828	0.576**	0.230
Nodes on primary branches vs. yield	0.276	0.211	0.162	0.926	0.821**	0.315

Pods on main stem vs. yield	0.838	0.532**	0.163	-0.984	-0.091	0.484**
Pods on branches vs. yield	0.720	0.757**	0.874**	0.921	0.872**	0.735**
Total pods vs. yield	0.847	0.859**	0.930**	0.943	0.899**	0.858**
Lodging vs. yield	0.052	0.044	0.000	0.607	0.549**	0.000

** Significant at 1% level, * Significant at 5% level.

G.B. Pant University of Agriculture and Technology
Department of Plant Breeding
Pantnagar, India

Visual selection for seed yield in standard plots in soybean.

Visual selection is the oldest form of plant breeding practiced by man. Its effectiveness when dealing with highly heritable characters in self-pollinated crops has been demonstrated repeatedly and thus visual selection for these characters is an accepted procedure. Although the effectiveness of visual discrimination for complex, less heritable characters, such as yield, is still debatable, visual selection is extensively utilized both on plant and row basis in carrying out selection in soybean. We, therefore, attempt to evaluate the value of visual scoring for classifying phenotypes for a complex character such as yield in a set of newly developed genotypes.

Materials and methods: Twenty-five newly developed breeding lines of soybean, including three checks ('Bragg', PK 327 and PK 416), were evaluated in a randomized block design with four replications in rainy season 1988. Each plot consisted of five rows, 4 m long and spaced at 60 cm. Within row spacing was about 5 cm. At the full podding stage, plots were phenotypically scored using scale from 1 to 5 (5 for most desirable, 1 for least desirable genotypes). Criteria preferred were large number of pods, resistance to lodging, disease and insects. Actual yields of three central rows were also measured.

Results and discussion: There was a positive correlation between seed yield and visual score (0.364). Although it was insignificant, it proved to be effective in identification of high-yielding lines visually. The details of visual score and seed yield (kg/plot ($7m_2$)) and other agronomic features are given in Table 1. Based on a visual score of 3.07 g lines PK 965, PK 966, PK 970, PK 971, PK 978, PK 982, PK 988, PK 416 were found to be phenotypically superior. However, on the basis of actual yield measurement, 14 lines (9 above lines and 5 additional ones i.e., PK 968, PK 969, PK 972, PK 987 and PK 327) were high

yielding. Thus, visual evaluation was successful in correct classification of 9/14 (64%) of the high yielding lines. On the contrary, 10/11 (90%) of poor yielding lines could be classified correctly based on phenotypic score. The success of visual selection has been reported by Byth et al. (1969), Garland and Fehr (1981), Pushpendra and Ram (1987) in soybean; Nass (1983), Yonezawa and Kato (1984) in wheat; Atkins (1964) in barley, and Ntare et al. (1984) in vigna.

The misclassification among high yielding lines was for 5 lines (PK 968, 969, 972, 987, 327). In all these lines the data showed that there was lodging. Therefore, in this experiment, lodging appeared to be the most important factor affecting our judgement. Hanson et al. (1962) reported misclassification due to lodging whereas Kwon and Torrie (1964) found that maturity and plant height had somewhat greater effect than that of lodging.

Conclusion: The positive correlation of phenotypic score with seed yield (0.36) indicated that visual evaluation was of value in identifying high yielding genotypes. Lodging was the most important factor affecting visual concept. Visual discrimination was effective in discarding 90% of the actually poor yielding lines and selecting 64% of the high yielding ones. Thus, visual discrimination utilizing information from replicated plots would be an effective criterion in soybean breeding programs, dealing with large numbers of breeding lines.

Score Scale: 1 least desirable
5 most desirable

Lodging scale: F: Free
M: Moderate
S: Susceptible

Correlation between seed yield and visual score: (r) = 0.364

Table 1: Average of maturity, plant height, lodging, visual score and yields/plot for all entries

Entries	Maturity (days)	Plant Height (cm)	Lodging	Visual score	Yield/ plot (kg/7.2 m^2)
PK 958	120.50	87.65	F	3.75	1.21
PK 965	116.75	97.10	F	4.00	1.50
PK 966	114.50	103.80	M	3.25	1.43
PK 968	115.25	81.55	M	2.50	1.36
PK 969	118.75	78.70	M	3.00	1.71
PK 970	118.75	80.20	F	3.50	1.65
PK 971	119.50	81.65	F	3.50	1.36
PK 972	119.25	110.00	M	2.00	1.37
PK 973	120.00	139.16	S	2.00	0.75
PK 974	121.25	154.35	S	2.00	0.87
PK 975	121.50	94.90	M	3.00	0.95
PK 976	122.50	96.4	S	3.00	1.21
PK 977	119.75	91.05	M	3.00	1.16
PK 978	119.25	94.75	F	3.50	1.44
PK 979	121.50	111.20	M	2.75	1.16
PK 981	115.75	103.80	S	2.75	1.12
PK 982	119.75	83.80	M	3.50	1.65
PK 983	116.56	87.70	M	3.00	1.22
PK 985	120.00	89.35	S	3.00	1.07
PK 987	123.00	97.90	M	3.00	1.07
PK 988	123.00	96.05	M	3.50	1.40
PK 990	121.75	133.40	S	3.00	0.96
PK 327	108.00	82.90	M	3.00	1.68
PK 416	120.25	87.75	F	4.00	1.65
Bragg	122.00	90.65	M	3.25	1.69
Average	119.12	98.17		3.07	1.31

References:

Atkins, R.E. 1964. Visual selection for grain yield in barley. Crop Sci., 4:494-496.

Byth, D.E., C.R Weber and B.E.Caldwell. 1969. Correlated truncation selection for yield in soybean. Crop Sci. 9:699-702.Garland, M.L. and W.R.Fehr. 1981. Selection for agronomic characters in hill and row plots of soybean. Crop Sci.; 21:591-595.

Hanson, W.D.; R.C.Leffel and H.W.Johnson. 1962. Visual discrimination for yield among soybean phenotypes. Crop Sci. 2:93-96.

Kwon, S.H. and J.H.Torrie. 1964. Visual discrimination for yield in two soybean populations. Crop Sci. 4:287-90.

Nass, H.G. 1963. Effectiveness of several selection methods for grain yield in two F^2 populations of spring wheat. Can. J. Pl. Sci. 163:61-66.

Ntare, B.R., M.E.Akenova, R.J.Redden and B.B.Singh. 1984. The effectiveness of early generation yield testing and single seed descent procedure in two cowpea (V.anguiculata (L.) Walp) crosses. Euphytica. 33:539-547.

Pushpendra and H.H.Ram. 1987. Studies on selection based on pod number, dry matter, harvest index, yield and visual superiority in F_6 generation of soybean, J. of Oilseeds Res., 4:289-291.

Yonezawa, K. and K.Kato. 1984. Efficiency of improving the precision of genotype assessment in selection of one plant with the uppermost selection score. Mem Coll. Agric. Kyoto Univ. 124:13-25.

P.T. Dao
Hari Har Ram

PUNJAB AGRICULTURAL UNIVERSITY
Department of Plant Breeding
Ludhiana, Punjab, India

Evaluation of soybean germplasm for resistance for yellow mosaic virus (YMV).

Soybean suffers from a number of diseases of which yellow vein mosaic, a viral disease, is most severe when under North-Indian conditions. The disease flourishes under high temperature and humidity and is transmitted by an aphid, Bamissia tabaci. Although indirect chemical methods of controlling the disease incidence by reducing aphid population are available, the cost-benefit ratio and chemical hazards involved indicate that the most desirable approach would be to exploit the genetic resistance. Identification of the source of resistance is a prerequisite step in this approach. Thus, an effort has been made to identify the source of resistance in soybean germplasm against the viral disease (YMV). Results are presented in the following text.

Materials and methods: Sixty genotypes of soybean, with diverse geographic origins, were evaluated under four environments over two years (1988 & 1989). Two sowing dates (end of June & first week of July) were observed with three replications in each environment. Evaluation was carried out under natural infection conditions. Plot size consisted of rows of 3 m length, spaced 45 cm apart. Disease data were expressed as percent plants infected and genotypes were classified for disease reaction into three groups: resistant (up to 15%), intermediate (15.1 to 40.0%) and susceptible ($\geq$ 40%).

Results and discussion: Pooled analysis of variance over environments (Table 1) indicated that mean squares, due to genotypes as well as environment, were significant. Genotype x environment interaction also was significant, showing that the genotypes differ in their response to environment for the disease reaction.

Table 1. Analysis of variance for virus incidence

Source	df	Mean squares
Genotypes	59	2149.68**
Environment	3	303.7**
Geno. & Environ.	177	58.9**
Error	472	0.565

**Significance at 1%

High heritability (89.7) and high genotypic coefficient of variation (41.6) indicated that the importance of additive gene action and direct selection will be helpful to identify the source of resistance. Out of 60 collections, none was found to be immune. The percent incidence varied from 13.3 to 67.9. On the basis of classification with respect to disease reaction, 6 entries were found to be resistant, 40 intermediate, and 14 susceptible (Table 2).

Table 2. Reaction of soybean genotypes to YMV.

S. No.	Reaction	No. of genotypes
1.	Resistant (up to 15%)	6
2.	Intermediate (15.1 to 40%)	40
3.	Susceptible (40.1%)	14

Genotype PK 416, a popular variety of soybean, had the lowest incidence and was closely followed by Himso-1548, SL 104, SL 160, and PK 564. In contrast, DS 81-1619 and DS 84-10 were highly susceptible.

The source of resistance identified in the above study can thus be effectively utilized to transfer resistance into the agronomically superior genotypes. Considering the high heritability of resistance to YMV, introgression of resistance seems to be amenable to simple back-cross methods or pedigree method of selection.

S.R. Sharma
P.S. Phul

JAPAN

HOKKAIDO UNIVERSITY
Faculty of Agriculture
Sapporo, 060, Japan

1) New electrophoretic mobility variants observed in wild soybean (Glycine soja) distributed in Japan and Korea

Isozymes are useful for evaluating genetic resources and also as genetic markers for chromosome mapping. In soybeans, more than 30 polymorphic loci have been identified for 20 enzyme systems. We have been examining allozyme variation of wild and cultivated soybeans in order to clarify their genetic structure and phylogenetic relationships. Several unreported electrophoretic mobility variants were observed in accessions of wild soybean collected in Japan and Korea. This paper reports characteristics of the observed variants, some of which may be helpful for understanding genetic basis of soybean isozymes.

Materials and methods: A total of 411 accessions, involving 383 from Japan and 28 from Korea (Republic of Korea), were assayed. Of these, 156 accessions from Japan were introduced from National Institute of Agrobiological Resources and National Agriculture Research Center, Tsukuba, Japan. The others were collected individually from natural habitats by our colleagues and us. For each accession, three germinated seeds were individually analyzed.

Nine enzyme systems were examined: aconitase (ACO), acid phosphatase (APH), diaphorase (DIA), endopeptidase (ENP), esterase (EST), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), mannose 6-phosphate isomerase (MPI), and phosphoglucomutase (PGM). Isozyme assays were adapted from Griffin and Palmer (1987), except as noted. We used two electrophoretic buffer systems Histidine-citrate (pH 6.5, D buffer by Cardy and Beversdorf, 1984) and Tris-Histidine (pH 7.0). The second buffer system, a modification of Second (1982), consists of 15 mM histidine HCl, 16 mM Tris pH 7.0 (gel buffer),

and 400 mM Tris, 132 mM citric acid HCl pH 7.0 (electrode buffer). Electrophoresis was conducted for five hours with a constant voltage of 250 V in the first system, and for six hours with a constant current of 25 mA in the second system. The enzymes visualized using the first system are ACO, APH, DIA and IDH. The enzymes visualized using the second system are PGM, MPI, ENP, EST and LAP. The staining procedure for these enzymes was adapted from Griffin and Palmer (1987) and Bult et al. (1989).

Results and discussion: Eleven unreported variants were observed in the six enzyme systems, ACO, APH, DIA, EST, lAP, and PGM (Fig. 1). The zymograms with the unreported variants all appeared to be homozygous because any intra-plant (between seeds) or any intra-accession variation was not detected when additional seeds from the same plant (accession) were assayed. The variants are characterized as follows:

Aconitase: ACO isozymes are controlled by at least five loci, Aco1, Aco2, Aco3, Aco4, and Aco5, each having two (Aco3-a, Aco3-b), three (Aco1-a, Aco1-b and Aco1-n, Aco2-a, Aco2-b and Aco2-c, Aco5-a, Aco5-b and Aco5-n), and four (Aco4-a, Aco4-b, Aco4-c, and Aco4-n) alleles (Doong and Kiang, 1987; Griffin and Palmer, 1987; Rennie et al., 1987; Kiang and Bult, 1991). Four unreported mobility variants, the two in Korea and the remaining two in both Korea and southern Japan, were observed (Fig. 1). Zymogram types 2 and 3 exhibited Band 1 migrating slower than Aco1-a. In Zymogram types 4 and 5 Band 2 migrating slightly slower than Aco3-a and in Zymogram type 6 Band 3 migrating faster than Aco3-b, each was substituted for Aco3-a or Aco3-b. Zymogram types 7 to 9 exhibited Band 4 migrating faster than Aco4-b and slower than Aco5-a, whereas they were absent from the functional alleles at either Aco4 (Zymogram type 7) or Aco5 (Zymogram type 8), or both (Zymogram type 9). Accordingly, Band 4 is considered to be controlled by different alleles at Aco4 and Aco5.

Acid phosphatase: APH isozymes are controlled by a single locus, Aph (Hildebrand et al., 1980). The three alleles (Aph-a, Aph-b and Aph-c) have been reported. An unreported mobility variant migrating faster than Aph-c was observed in an accession

(B00040) from Korea (Fig. 1).

Diaphorase: DIA isozymes are controlled by at least three loci, Dia1, Dia2, and Dia3, each having two alleles (Dia1-a and Dia1-b, Dia2-a and Dia2-b, Dia3 and dia3) (Gorman et al., 1983). Dia1 controls the expression of a cluster of five bands. Gorman et al. (1983) suggested from the analysis of variation in Glycine clandestina that this cluster was controlled by two loci, with the middle bands being heterotetramers and the slowest and the fastest migrating bands homotetramers from these loci.

Two unreported variants were observed for Dia1 (Fig.1). Zymogram type 3, which was observed in an accession (B00040) from Korea, exhibited a cluster of five bands as Dia1-a (Zymogram type 1), with each band migrating slower than each of the counterparts formed by Dia1-a. Zymogram type 4, observed in three accessions (B02009, B03004 and B04085) from northern Japan, did not exhibit these clusters, in which only the three fast migrating bands were observed in high intensity. These variants may also be explained by assuming the two loci with codominant and recessive null alleles. In Zymogram type 4, one of the two loci involved (tentatively designated Dia4) is considered to have a recessive null allele, so that the slowest migrating band may be not visible, while the other bands may increase in intensity with mobility. The putative genotype for each zymogram is presented in Fig. 1.

Esterase: Cathodal EST isozymes are controlled by a single locus, Est (Bult and Kiang, 1989). Two alleles (Est-a and Est-b) have been reported. A null form, absent from the EST activity, was observed in an accession (B07004) from Tokushima Prefecture, Shikoku, Japan (Fig. 1).

Leucine aminopeptidase: LAP isozymes are controlled by two loci, Lap1 and Lap2, each having two alleles (Lap1-a and Lap1-b, Lap2 and Lap2) (Kiang et al., 1985, Kiang and Chiang, 1987). An unreported variant migrating slightly faster than Lap1-b was observed in five accessions (B01134, B01135, B01136, B01141 and B01147) from Hokkaido, Japan (Fig. 1).

Phosphoglucomutase: PGM isozymes are controlled by two loci, Pgm1 and Pgm2 (Gorman et al., 1983). Two alleles (Pmg1-a and Pmg1-b) have been reported for Pgm1. Two alleles responsible for the expression of Bands 2 and 3 are observed in Pgm2, but no allele symbols have been given for them. Two genetic hypotheses are presented for the type of alleles (Gorman et al., 1983). The first is that the two alleles interact in codominant fashion, each coding bands with different mobility. The second is that this locus is polymorphic for dominant functional and recessive null alleles, with a regulatory effect on the expression of Band 3 with the alleles.

Two unreported mobility variants, the one (Band 1) migrating slower than Band 2 and the other (Band 4) migrating faster than Band 3, were observed for Pgm2 (Fig. 1). Zymogram types 5 and 6 exhibited Bands 1 and 3, whereas Zymogram type 7 exhibited Band 2 and 4. Zymogram type 8 has only Band 2, and zymogram type 9 has Bands 1 and 2. These newly found variants may support the two-loci hypothesis with codominant alleles for explaining the genetic basis of the observed variation. Pgm2 may contain three codominant alleles, each coding Bands 1,2 and 3, whereas an additional locus, tentatively designated Pgm3, may code bands 3 and 4. Accordingly, Band 3 is considered to be controlled by different alleles at Pgm2 and Pgm3, as in the case of Idh1-b and Idh2-a alleles (Kiang and Gorman, 1985). The putative genotype for each zymogram is presented in Fig. 1. However, it is not clear from the data whether these loci have a recessive null allele or not. Band 1 was observed in ten accessions from Korea and Japan, and Band 4 in five accessions, the one (B00049) from Korea and the four (B09014, B09015, B09028 and B09029) from Kyushu, Japan.

Geographic distribution of the observed variants: Frequencies of the observed variants ranged from 0.3% to 2.6%, most of them being lower than one percent. These variants have not yet been observed in about 1000 accessions of cultivated soybean from Japan, Korea, Taiwan and China which we assayed so far. Accordingly, these variants are unique to wild soybean,

suggesting its rich genetic diversity.

Geographic distribution of the variants is summarized in Fig. 2. Eight of the 11 variants were distributed regionally. Of these, four were observed in accessions from Korea, one from Korea and Kyushu, the remaining three each from Hokkaido, Honshu (Tohoku, Kantou and Hokuriku), and Shikoku. Thus, our data indicate that Korea may be one of the centers of wild soybean gene diversity. Our findings also suggest that the other regions may also form their own gene pools, where uncommon variants have been accumulated independently and gradually.

Acknowledgments: The assistance of H. Konishi and T. Iida is gratefully acknowledged. This work was supported in part by Grant-Aid from the Ministry of Education, Science and Culture, Japan and by a grant from Japan Beans and Peas Foundation.

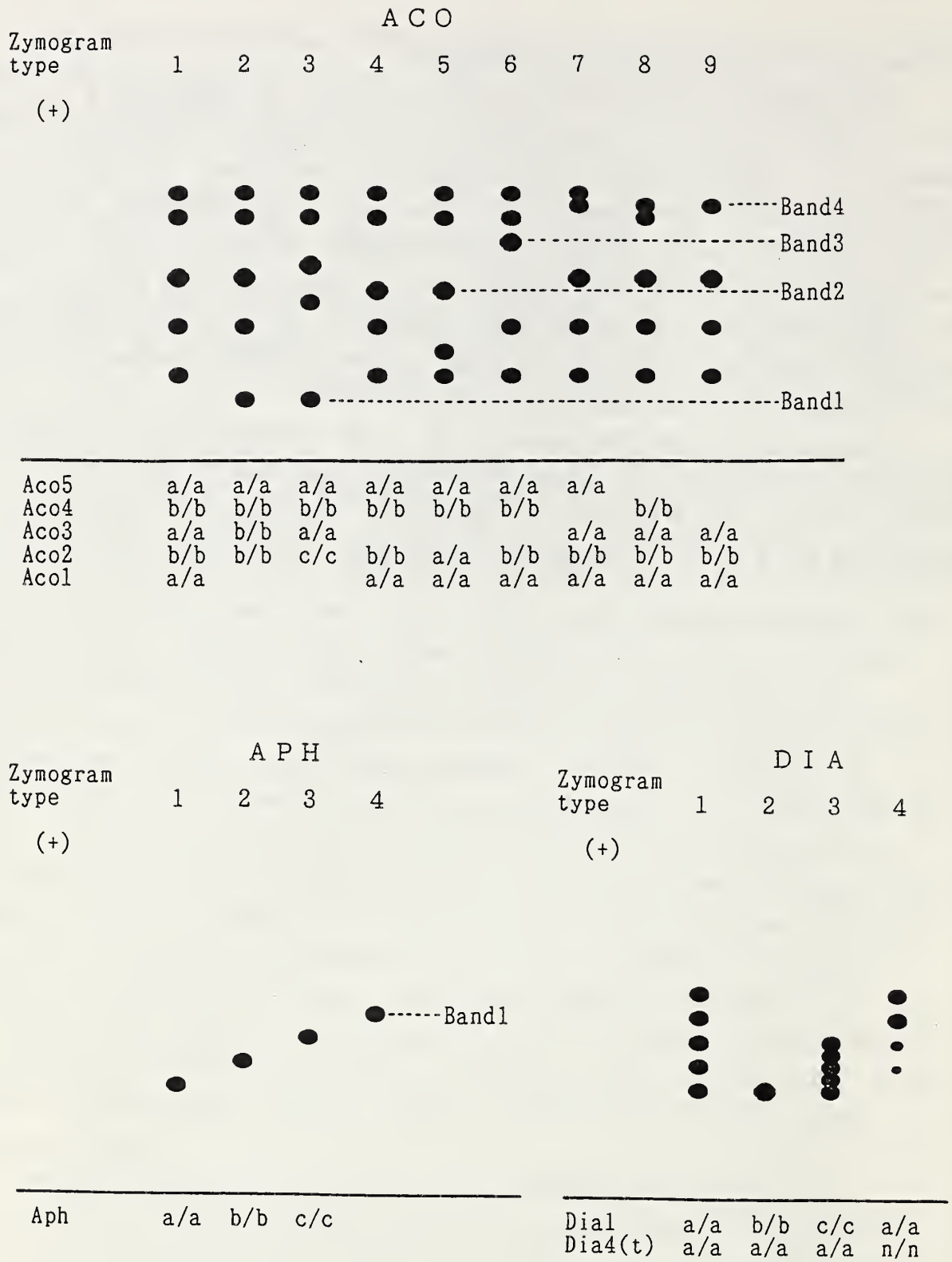
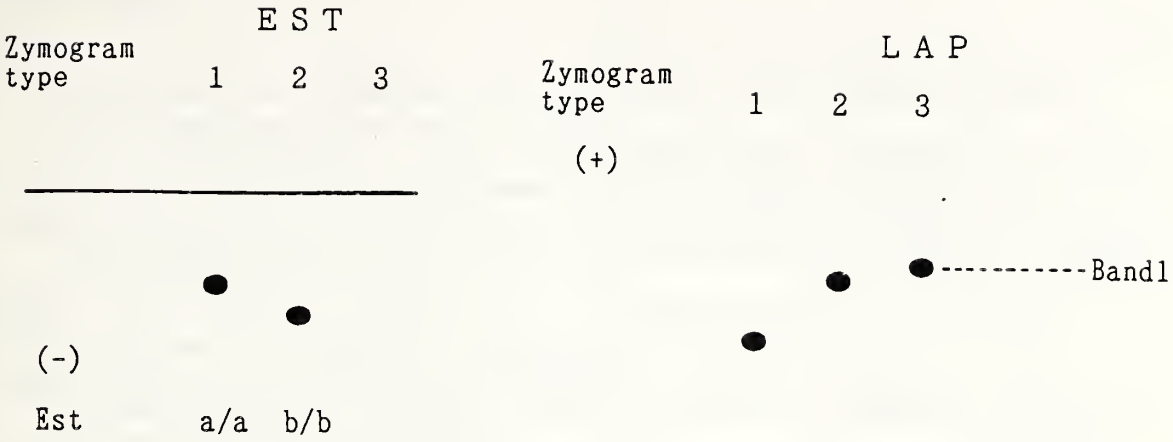
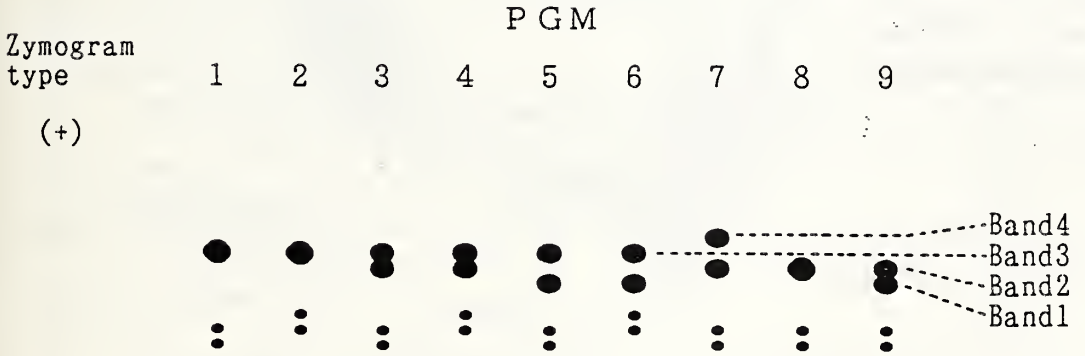


Fig.1 Electrophoretic mobility variants newly observed in wild soybean.





Pgm3(t)	b/b	b/b	b/b	b/b	b/b	b/b	c/c	a/a	a/a
Pgm2(t)	c/c	c/c	b/b	b/b	a/a	a/a	b/b	b/b	a/a
Pgm1	a/a	b/b	a/a	b/b	a/a	b/b	a/a	a/a	a/a

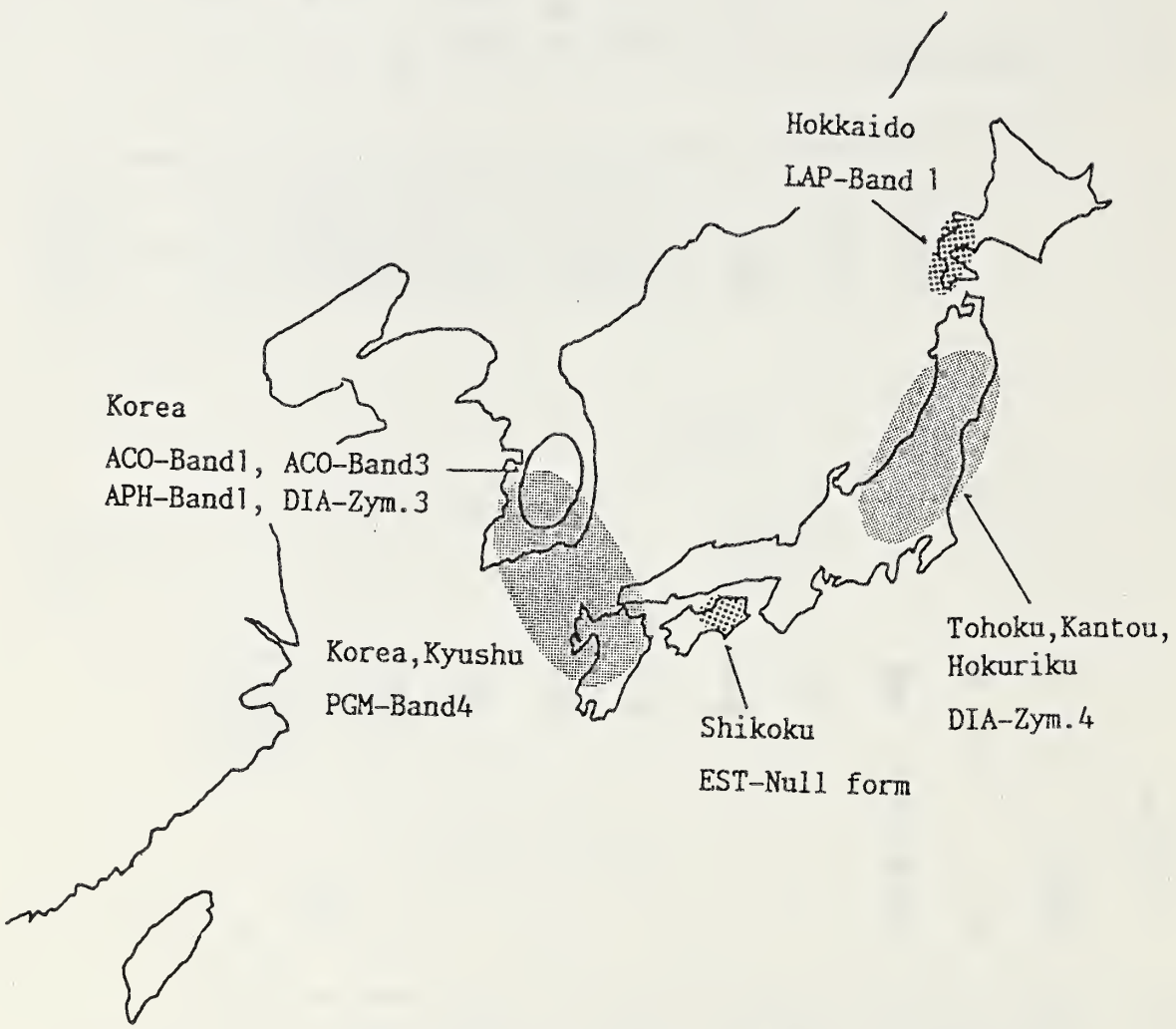


Fig.2 Geographic distribution of the unreported variants observed in wild soybean from Japan, Korea.

Bands 2 and 4 for ACO and Band 1 for PGM were observed in accessions from Korea and Japan (Kyushu, Kinki, Tokai, and Kantou).

References

Bult, C.J. and Y.T.Kiang. 1989. Inheritance and genetic linkage tests of an esterase locus in the cultivated soybean, Glycine max. J.Hered. 80:82-85

___, ___, M.B.Gorman. 1989. Electrophoretic methods for soybean genetics studies. Soybean Genet. Newsl. 16:175-184.

Cardy, B.J. and W.D.Beversdorf. 1984. Identification of soybean cultivars using isozyme electrophoresis. Seed Sci.Technol. 12:943-954.

Doong, J.Y. and Y.T.Kiang. 1987. Inheritance of aconitase isozymes in soybean. Genome 29:713-717.

Gorman, M.B., Y.T.Kiang, R.G.Palmer and Y.C.Chiang. 1983. Inheritance of soybean electrophoretic variants. Soybean Genet. Newsl. 10:67-84.

Griffin, J.D. and R.G.Palmer. 1987. Inheritance and linkage studies with five isozyme loci in soybean. Crop Sci. 27:885-892.

Hildbrand, D.F., J.H.Orf and T.Hymowitz. 1980. Inheritance of an acid phosphatase and its linkage with the Kunitz-trypsin inhibitor in seed protein of soybeans. Crop Sci. 20:83-85.

Kiang, Y.T. and M.B.Gorman 1985. Inheritance of NADP-active isocitrate dehydrogenase isozymes in soybeans. J.Hered. 76:279-284.

___ and Y.C.Chiang. 1987. Genetic analysis of the Lap2 locus in soybeans and tests of linkage with other loci. J.Hered. 78:131-132.

___ and C.J.Bult. 1991. Genetic and linkage analysis of aconitate hydratase variants in soybean. Crop Sci. 31:322-325.

___, M.B.Gorman and Y.C.Chiang. 1985. Genetic and linkage analysis of a leucine aminopeptidase in wild and cultivated soybean. Crop Sci. 25:319-321.

Rennie,B.D., W.D.Beversdorf and R.I.Buzzell. 1987. Genetic and linkage analysis of an aconitate hydratase variant in the soybean. J.Hered. 78:323-326.

Second, G. 1982. Origin of the genetic diversity of cultivated rice (Oryza spp.): study of polymorphism scored at 40 isozyme loci. Jpn. J. Genet. 57:25-57.

J. Abe
M. Ohara
Y. Shimamoto

2. Glycine soja germplasm in Japan: Isozyme and chloroplast DNA variation

Glycine soja is considered to be a wild progenitor of cultivated soybean G. max because of easy crossability, wide phenotypic overlap and geographical propinquity. The phylogenetic relationships between these two species have recently been the focus of biochemical studies based on protein and DNA comparisons (Kiang et al., 1978; Shoemaker et al., 1986; Doyle, 1988; Close et al., 1989). We have initiated isozyme and chloroplast (cp) DNA analyses of G. soja germplasm collection in order to trace genetic diversification and to produce the rational phylogenetic scheme for a given taxon. Preliminary results are presented here.

Germplasm accessions: Table 1 lists the soybean cultivars and G.soja accessions used in this study. G.soja seeds were collected from 25 local populations in Japan (Fig. 1).

Isozyme variation: Five enzyme systems, representing at least 7 loci, were assayed (Table 1): aconitase (ACO), acid phosphatase (APH), diaphorase (DIA), endopeptidase (ENP) and isocitrate dehydrogenase (IDH). Four to 7 plantlets per accession were investigated. The Idh2 locus was found to be monomorphic for all plants surveyed, whereas all others had 2 or 3 alleles. Our results also revealed isozymically heterogeneous accessions; in 9 out of 119 cases (loci x accessions) there was polymorphism. Comprehensive analysis of isozyme patterns would be useful for determination of the genetic distances among accessions of G.max and G.soja.

cpDNA polymorphism: Total DNA was isolated from greenhouse-grown seedlings. The DNA was subjected to complete digestion with EcoRI or ClaI enzyme, followed by gel electrophoresis and filter transfer.

Close et al. (1989) recently reported sufficient cpDNA sequence diversity within the subgenus Soja to delineate six groupings based upon restriction fragment length polymorphisms (RFLPs). Three out of 6 fragment polymorphisms detected proved to be due to changes in the large single-copy area near the

junctures with the left member of the inverted repeat. The molecular conservatism of the land plant chloroplast genomes facilitates the use of heterologous probes to screen G.soja genotypes for their cpDNA differences.

In this study, EcoRI or ClaI digests of G.max and G.soja DNAs were probed with a sugarbeet H2 clone (T. Mikami et al., unpublished) which occupies the juncture region between the left member of the inverted repeat and the large single-copy region of cpDNA molecules. Table 1 summarizes the RFLP data. The 4.8-kbp (cv.'Minsoy') and 2.5-kbp (cv.'Peking') EcoRI polymorphic fragments were identified with the sugarbeet probe, a pattern that agrees well with the previous observation (Close et al., 1989). Hybridization of ClaI digested DNAs to the same probe also detected the mutation resulting in a 2.4-kbp fragment in Minsoy versus a 3.5-kbp fragment in Peking.

The germplasm survey revealed two groups of G.soja cpDNA; most of the accessions that shared RFLP patterns with Peking; the four populations from Shimane district displayed RFLP profiles indistinguishable from that characteristic of Minsoy (a modern cultivar). Similar results were reported by Close et al. (1989), who could not differentiate three G.soja Plant Introductions from Minsoy. It seems likely that the Shimane accessions examined are of G.max x G.soja hybrid origin and that their cytoplasmic donor is G.max, though the definite proof remains to be seen.

Acknowledgements: We thank National Institute of Agrobiological Resources Japan for supplying seed samples. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan and by a grant from Japan Beans and Peas Foundation.

Table 1. Summary of isozyme and cpDNA analyses of G. soja germplasm

No.	Accession Cultivar	Origin	Distribution of alleles*							PFLPs in cpDNA Polymorphic frag.	
			Aco2	Aco3	Aph	Dia1	Enp	Idh1	Idh2	EcoRI	ClaI
G. soja											
1	B01083	Hokkaido	b	a	c	a	a	b	a	2.5	3.5
2	B01089	Hokkaido	b	a	p(a+b)	a	a	b	a	2.5	3.5
3	B01125	Hokkaido	b	a	c	a	b	b	a	2.5	3.5
4	B01132	Hokkaido	b	a	c	a	b	b	a	2.5	3.5
5	B01137	Hokkaido								2.5	3.5
6	B01134	Hokkaido	p(a+b)	a	p(a+c)	a	b	b	a	2.5	3.5
7	B01141	Hokkaido	b	a	a	b	b	b	a	2.5	3.5
8	B02028	Aomori								2.5	3.5
9	B02032	Aomori								2.5	3.5
10	B02002	Aomori	b	a	c	a	b	b	a	2.5	3.5
11	B02044	Aomori								2.5	3.5
12	B02004	Aomori	b	a	b	b	b	b	a	2.5	3.5
13	B02005	Aomori	b	a	b	b	b	b	a	2.5	3.5
14	B02007	Akita	b	a	c	b	b	b	a	2.5	3.5
15	B02057	Iwate								2.5	3.5
16	B02059	Iwate								2.5	3.5
17	B02026	Fukushima	b	p(a+b)	b	p(a+b)	a	a	a	2.5	3.5
18	B06002	Wakayama	b	a	c	a	a	b	a	2.5	3.5
19	B06004	Hyogo	b	a	c	b	a	b	a	2.5	3.5
20	B08003	Okayama	b	a	c	b	b	b	a	2.5	3.5
21	B08006	Shimane								4.8	2.4
22	B08007	Shimane	b	a	b	a	a	b	a	4.8	2.4
23	B08008	Shimane								4.8	2.4
24	B08009	Shimane	p(a+b)	a	b	b	p(a+b)	b	a	4.8	2.4
25	B09002	Kumamoto	b	a	b	b	p(a+b)	p(a+b)	a	2.5	3.5
G. max											
26	Minsoy									4.8	2.4
27	Peking									2.5	3.5

* p stands for polymorphism in the cases where different individuals have different alleles.

** Polymorphic fragments were detected by Southern analysis using the sugarbeet H2 clone probe. Fragment sizes are given in kbp.

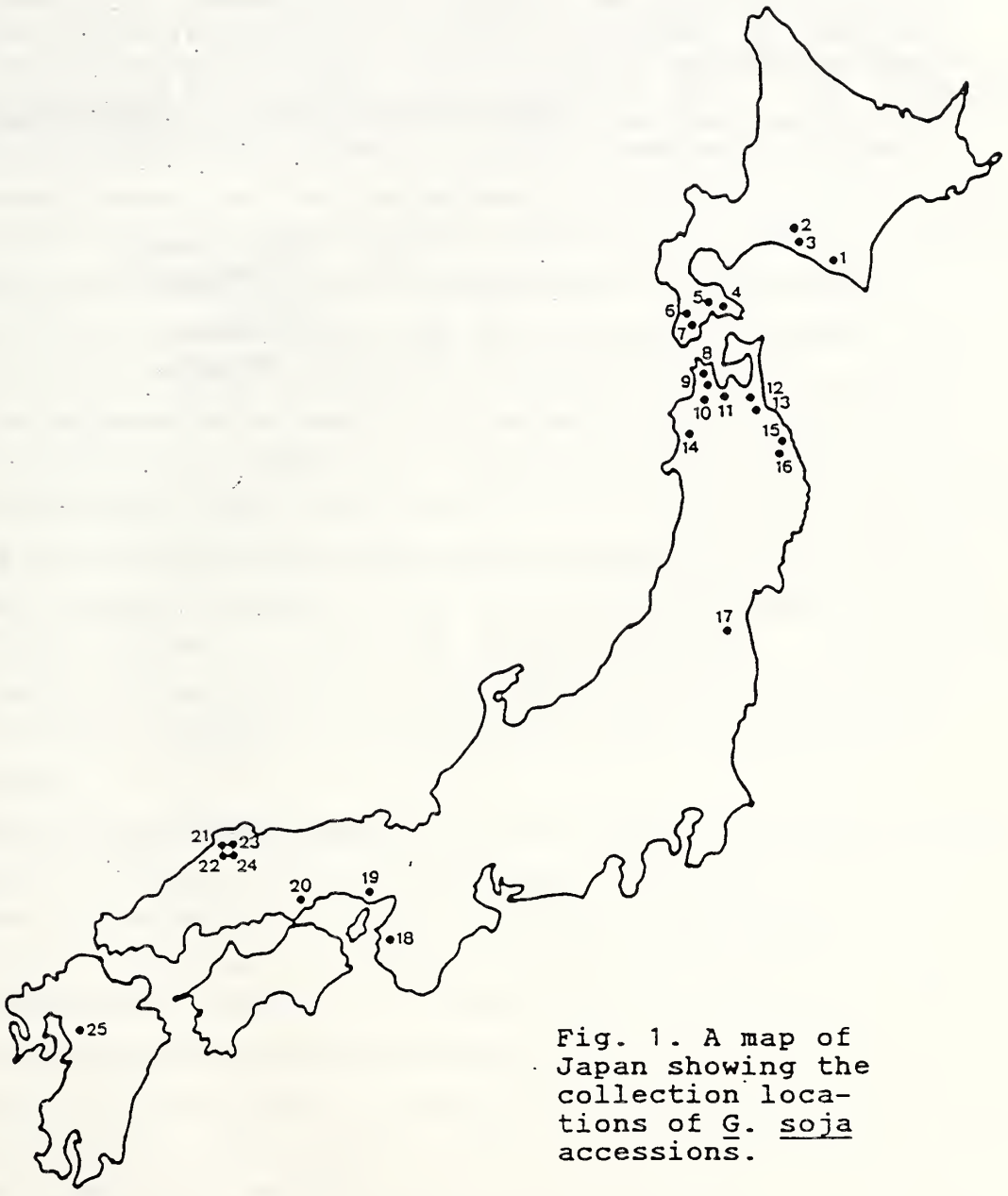


Fig. 1. A map of Japan showing the collection locations of G. soja accessions.

References

Close, P.S., R.C.Shoemaker and P.Keim. 1989. Distribution of restriction site polymorphism within the chloroplast genome of the genus Glycine, subgenus Soja. Theor. Appl. Genet. 77:768-776.

Doyle, J.J. 1988. 5s ribosomal gene variation in the soybean and its progenitor. Theor. Appl. Genet. 75:621-624.

Kiang, Y.T., Y.C.Chiang, J.Y.H.Doong and M.B.Gorman. 1987. Genetic variation of soybean germplasm. In: S.C. Hsieh [ed] Crop Exploration and Utilization of Genetic Resources. Published by Taichung District Agricultural Improvement Station, Taiwan.

Shoemaker, R.C., P.M. Hatfield, R.G. Palmer and A.G.Atherly.1986. Chloroplast DNA variation in the genus Glycine subgenus Soja. J.Hered. 77:26-30.

Y.Shimamoto
A.Hasegawa
J.Abe
M.Ohara
T.Mikami

POLAND

AGRICULTURE UNIVERSITY
Faculty of Agriculture
Department of Genetics and Plant Breeding
60-625 Poznan ul. Wojska Polskiego 71 C

Variation and heritability of phenological characteristics of soybean genotypes in Polish conditions:

Introduction: Photosensitivity and thermic and water requirements determine the usefulness of soybean genotypes for cultivation in given environmental conditions. Specific reaction of soybean genotypes to Polish geographic and meteorologic factors, like long daylength, spring and autumn coolness etc., need to be elucidated. Research on genetic and environmental impact on expression of some more important traits should be done. Recognized variation and heritability of soybean in the Polish environment may contribute to more efficient evaluation of breeding material and may hasten the release of new cultivars. There are not many studies available on solving the problems in introducing soybean to north and moderate climate. In this study some attempts to measure variation and heritability of highly differentiated genotypes of soybean were made in the Polish environment.

Plant material and methods: In the study, 25 differentiated genotypes of soybean selected from the Collection of the Department of Genetics and Plant Breeding, Agricultural University, Poznan, were under consideration (Table 1). The experiments were carried out in 1976, 1977, and 1979 in the field of the Agricultural Experiment Station at Swadzim near Poznan. Experiments were arranged in partially-balanced quadratic lattice design with three replications. The spacing between plants was 10x30 cm. Two seeds per hill were sown manually. Plots were 1.2x1.5 m with four rows. After emerging seedlings were thinned to one plant per hill.

Phenological observations which, in a certain way, are

associated with or may be important in selection and breeding, were considered. Statistical calculations were performed on the values of morphologic traits taken from the whole plots. Data were used for statistic calculation by means of multivariate analysis of variance. Heritability of traits was calculated as relation of genotypic variance to phenotypic variance.

Results and discussion: Period from sowing to plant emergence depended on temperature and water availability. The longest period for emergence was observed in 1979 when daily temperature oscillated around 10°C and there was only 6.8 mm of rainfall (Table 2). Such conditions extended the period from sowing to emergence up to 20 days, i.e., 15.2% of the whole vegetative period. In 1977, the weather was more favorable for emerging plants and soybean emerged early, 12.7 days after sowing. Above mentioned dependence showed that soybean in the Polish environment should be sown when temperature is above 10.0°C. Sowing soybean too early may cause losses in plant density. Similar conclusions about sowing date and emergence were found by Jaranowski et al.(1981).

On average, the period from emergence to the beginning of flowering took about 32% of the whole vegetative period of examined genotypes (Table 3). There were substantial differences observed among years of experimentation (Table 2). In 1976 and 1977, the period from emergence to flowering took more than 34% and in 1979 it took 27.4% of the whole vegetative period. It seems that such differences were due to hot weather after plant emergence in 1979 as compared to other years. Relatively wet, cool conditions in 1977 did not reduce that effects of that period.

Among examined phenologic characteristics (after plant emergence) the duration of flowering was the shortest period (Table 2 and 3). On average, it took about 17% of the whole vegetative period (Table 3). In 1976, the temperature during flowering was exceptionally high compared to typical Polish conditions, but mean duration of flowering was longer than in 1979 when the temperature was lower. It proves that temperature

during foregoing periods influenced duration of flowering as well. When particular genotypes and years were considered, the duration of the flowering period was highly differentiated: from nine to 57 days (Table 3). Furthermore, its coefficient of variation was the highest and this trait differentiated the genotype most. In spite of the high coefficient of variation the heritability of flowering period was very high (Table 3).

The period of pod filling took almost 39% of the whole vegetative period of soybean (Table 3). For this trait the range and coefficient of variation and heritability were moderate when compared with other phenologic traits. Comparing duration of periods foregoing pod filling with pod filling period it was evident that there was negative relation among them. In 1976 and 1977, pod filling periods were relatively short and foregoing periods were longer (Table 2). In spite of relatively high temperature in 1979 during pod filling period it was long when compared with preceding periods in which the temperatures were high as well.

The whole vegetative period of examined genotypes showed a wide range of 109-171 days. These characteristics showed the smallest coefficient of variation (9.5%) and the greatest heritability (0.92) among all characteristics examined (Table 3). Similar results were obtained in the studies on soybean collection by Jaranowski et al. (1983) and Skorupska et al. (1986). In these studies the 113 genotypes of soybean showed a narrower range of vegetative period from 124 to 153 days and coefficient of variation of this characteristic was 10.4%.

'Fiskeby V' cultivar was distinguished by its short vegetation period and the other phenological stages. It was highly insensitive to changing weather conditions in consecutive years. In 1979 it matured 109 days from sowing.

Independently from the duration of vegetation period in the consecutive years of studies the total sums of daily temperature for these whole vegetative periods were close to each other (Table 2). It seems that soybeans need rather constant temperature to mature successfully, especially in north latitudes.

Table 1. Soybean genotypes selected out for experimentation.

Number	Genotype	Maturity group	Origin
1	PI 250.002	00	Belgium
2	Altona	00	Ontario, Canada
3	PI 153.299	00	Belgium
4	Mazowiecka, II	0	Poland
5	PI 291.320 B	0	China
6	Tiara	I	Unknown
7	PI 257.431	00	Germany
8	Herb - 622	I	Unknown
9	PI 154.193	00	Holland
10	Vansoy	0	Ontario, Canada
11	PI 180.524	00	Germany
12	PI 238.923	00	Czechoslavakia
13	PI 161.431 B	00	Sweden
14	Warszawska	0	Poland
15	PI 332.899	0	Hungary
16	Norchief	0	USA
17	PI 231.172	00	Sweden
18	PI 180.508	00	Germany
19	Amurskaya	I	Russia
20	Fiskeby V	00	Sweden
21	PI 196.525	00	Sweden
22	PI 232.997	00	Germany
23	PI 189.900	0	France
24	PI 196.526	00	Sweden
25	PI 179.822	0	Germany

Table 2. Weather conditions in consecutive soybean phenophases of growing seasons: 1976, 1977, and 1979.

Year	1976			1977			1979		
Period	a °C	b mm	c days	a °C	b mm	c days	a °C	b mm	c days
From sowing to emergence	11.4	8.8	16.6 11.6% +	14.2	24.2	12.7 8.8%	10.1	6.8	20.0 15.2%
From emergence to flowering	14.6	47.9	49.7 34.7%	13.8	78.7	49.6 34.3%	18.3	52.6	36.2 27.4%
Flowering #	19.0	96.9	23.4 16.3%	16.5	106. 2	30.6 21.1%	17.1	22.1	17.9 13.6%
Pod filling $	14.9	116. 2	53.6 37.4%	15.3	130. 4	51.8 35.8%	16.5	73.1	57.7 43.8%
Vegetative *	15.0	269. 8	143.3 100%	15.0	339. 5	144.7 100%	15.6	154. 6	131.8 100%
Total temp.		2162			2178			2137	

a = Daily mean temperature
b = Sum of rainfall
c = Mean length of given period (over all genotypes)
# = From begin to end of flowering
$ = From end of flowering to harvest maturity
* = From sowing to harvest maturity
+ = Percent of the whole vegetative period

Table 3. Phenological characteristics and their means, ranges of variation, coefficients of variation (V) and heritability (h^2) over genotypes and years of experiment (1976, 1977, and 1979).

Period	Mean [days]	Range [days]	V [%]	h^2
From emergence to flowering	45.2 32.3%+	24-69	19.0	0.80
Flowering #	24.0 17.1%	9-57	42.1	0.87
Pod filling$	54.4 38.8%	36-82	14.8	0.58
Vegetative*	139.9 100%	109-171	9.5	0.92

From beginning to end of flowering
$ From end of flowering to harvest maturity
* From sowing to harvest maturity
+ Percent of the whole vegetative period

Table 4. Means ($\bar{X}$) of vegetative period and coefficients of variation (V) of particular genotypes over 1976, 1977, and 1979.

Number	Genotype	Vegetative period $\bar{X}$	V[%]
1	PI 250.002	135	1.2
2	Altona	138	2.0
3	PI 153.299	143	0.8
4	Mazowiecka II	136	0.3
5	PI 291.320B	146	0.1
6	Tiara	139	1.6
7	PI 257.431	141	1.7
8	Herb - 622	124	2.0
9	PI 154.193	156	2.5
10	Vansoy	160	1.8
11	PI 180.524	137	1.2
12	PI 238.923	128	2.8
13	PI 161.431B	132	1.0
14	Warszawska	126	2.4
15	PI 332.899	152	0.4
16	Norchief	165	1.8
17	PI 231.172	134	5.9
18	PI 180.508	142	1.4
19	Amurskaya	153	1.7
20	Fiskeby V	120	2.5
21	PI 196.525	150	2.9
22	PI 232.997	139	1.4
23	PI 189.900	141	1.5
24	PI 196.526	127	3.6
25	PI 179.822	137	1.6

References:

Jaranowski J., G. Konieczny, and J. Nawracala. 1981. Wzrostirozwoj soi (Glycine max (L.) Merr.) w. Zaleznosci od wczesnowiosennych siewow (Growth and development of soybean (Glycine max (L.) Merr.) dependent on early sowing date). Prace Kom. Nauk Roln. i Kom. Nauk Lesnych. LI;83-96.

___, H. Skorupska, G. Konieczny, A. Muszynski, and L. Torz. 1983. Charakterystyka zmiennosci wazniejszych pod wzgledem gospodarczym cech uzytkowych soi (Characteristics of variability of some economically important useful features of soybean). Zesz. Probl. Post. Nauk Roln. 253;7-24.

Skorupska H., J. Nawracala, and G. Konieczny. 1986. Prace genetyczno-hodowlane nad soja Glycine max (L.) Merr. (Genetic and breeding research on soybean Glycine max (L.) Merr.). Roczniki Akademii Rolniczej w Poznaniu. CLXVIII;137-145.

Grzegorz Konieczny
Adam Muszynski
Jerzy Nawracala

UNITED STATES

IOWA STATE UNIVERSITY
Department of Agronomy
Ames, IA 50011

1) Ovule abortion study in four partial-sterile soybean mutants

PS-1, PS-2, PS-3 and PS-4 are four independent mutations for partial sterility discovered in different F^{11} families, each of which had descended from a different F^{9} plant of the Asgrow Mutable line. PS-1 is true breeding for partial sterility and PS-2, PS-3 and PS-4 segregate for normal and partial sterile plants. These mutations were recovered from a transposon tagging study and provide evidence for transposition of a mobile genetic element (Groose and Palmer, 1987).

Generally transposition of a mobile genetic element causes chromosomal rearrangements, such as translocations, inversions, deletions or base substitutions.

The objective of this research was to determine whether a relationship exists between the position of an ovule or seed abortion in the pod and chromosome structure in PS-1, PS-2, PS-3, and PS-4 soybean mutants.

Materials and methods: Seeds from four mutants were field planted. In each row, five randomly selected plants were tagged at flowering. At maturity, plants were classified as normal or partial sterile, lifted and brought into the laboratory. A pod-by-pod record of seed, aborted seed and aborted ovules was made for all plants. A total of 60 PS-1 plants, 80 PS-2 plants, 50 PS-3 plants and 35 PS-4 plants were scored over 1990 and 1991.

A code designation was established to related individual seed to position in the pod according to Palmer and Heer (1984). Percentage of ovule abortion for two-ovule and three-ovule pods were calculated for each mutant. These studies were conducted in summer 1990 and 1991, Ames, Iowa.

In 1990, flowers were collected into stained 70% ethanol and

classified for fertility/sterility. Pollen was classified with a solution of I_2KI, and examined under a microscope.

Results and discussion: Table 1 shows the percentage of ovule/seed abortion for each of the four partial steriles in both years. There is not any great difference among the four partial steriles in two-ovule pods and three-ovule pods. For ovaries with two ovules, the range of ovule abortion ranged from 29.5% to 34.1% and 32.1% to 35.6% for 1990 and 1991, respectively. More abortions in three-ovule pods were observed in 1990 data, suggesting that the environment was less favorable.

In Table 2, the average number of pods per plant with respect to seed position in the pod, seed abortion and ovule abortion are presented. In the partial sterile plants for all four mutants, there were about equal frequencies of ovule abortion with respect to position in the ovary within two-ovule pods and within three-ovule pods.

In the normal plants from partial sterile families, there were few ovule abortions, but most of them occurred in the basal position. This result agrees with the results obtained by Palmer and Heer (1984).

The observations of pollen stained with I_2KI indicated no difference between normal and partial sterile genotypes. That means that all pollen grains from normal and partial sterile plants were plump and stained red-brown, presumably meaning fertile pollen.

Based on these results, we believe that we are dealing with mutants that cause ovule abortion, because we have a random distribution of abortion of ovules in partial sterile plants, like Palmer and Heer (1983) had working with a heterozygous interchange plants. Genetic and cytogenetic studies are being conducted.

Table 1. Percentage of ovule abortion in four partial sterile soybean mutants for two years.

Mutant	No. plants		Ovaries with 2 ovules		Ovaries with 3 ovules	
	1990	1991	1990	1991	1990	1991
Partial Sterile #1	30	30	34.1	34.9	44.2	41.5
Partial sterile #2						
Normal plants	14	32	17.8	21.3	25.1	17.2
Partial steriles	16	18	33.6	32.1	43.9	43.1
Partial sterile #3						
Normal plants	17	12	22.0	19.8	27.0	15.9
Partial steriles	8	12	31.1	32.9	46.6	43.2
Partial sterile #4						
Normal plants	5	17	15.5	15.7	19.6	14.0
Partial steriles	5	8	29.5	35.6	46.8	41.7

Table 2. Distribution of the number of seeds, seed abortions and ovule abortions per pod for Partial Sterile #1 (PS1), #2 (PS2), #3 (PS3), and #4 (PS4) soybean mutants for two years.

Mutant	Type of pod *									
	++	-+	+-	+++	-++	++-	-+-	+-+	--+	+--
Partial Sterile #1										
1990	31.7	34.7	33.5	9.3	18.3	10.3	9.6	20.1	13.6	18.8
No. of plants= 30										
1991	30.1	31.5	38.3	15.7	15.1	15.9	14.9	13.1	13.8	11.3
No. of plants= 30										
Partial Sterile #2										
1990										
SS	32.8	37.9	33.5	13.3	17.1	12.3	17.9	12.1	16.9	10.3
No. of plants= 16										
N	64.2	18.9	16.8	40.2	38.5	2.8	2.4	3.0	12.6	0.4
No. of plants= 14										
1991										
SS	35.7	32.7	31.6	12.1	19.3	13.3	14.6	14.0	16.5	10.2
No. of plants=18										
N	57.4	33.6	9.0	57.5	22.7	5.9	1.7	4.8	6.5	0.8
No. of plants=32										

Partial Sterile #3										
1990										
SS	37.7	38.8	23.4	11.3	16.1	9.6	17.3	12.0	19.0	14.6
No. of plants=8										
N	55.9	40.9	3.2	37.2	37.8	2.7	2.8	4.1	14.9	0.4
No. of plants=17										
1991										
SS	34.1	34.7	31.1	12.2	18.0	13.5	13.9	14.2	16.3	11.7
No. of plants=13										
N	60.3	25.0	14.7	58.9	20.8	6.3	2.8	4.2	6.3	0.7
No. of plants=12										
Partial Sterile #4										
1990										
SS	40.9	30.1	28.9	9.9	16.7	10.2	18.1	12.6	20.5	11.9
No. of plants=5										
N	69.0	26.8	4.2	51.4	27.9	5.8	2.1	4.5	6.5	1.6
No. of plants=5										
1991										
SS	28.8	39.4	31.8	14.9	15.1	17.0	15.1	12.8	15.3	9.7
No. of plants=8										
N	68.5	22.2	9.2	63.7	22.4	5.0	1.7	3.2	3.4	0.5
No. of plants=17										

* A mature seed is represented as +, and ovule or seed abortion as -; e.g., -++ means abortion (seed or ovule) in the basal position, a seed in the middle and apical postions.

References

Groose, R.W. and R.G. Palmer. 1987. New mutations in a genetically unstable line of soybean. Soybean Genet. Newsl. 14:164-167.

Palmer, R.G. and H.E. Heer. 1984. Agronomic characteristics and genetics of a chromosome interchange in soybean. Euphytica 33:651-663.

T.N.S. Pereira
R.G. Palmer - USDA

2) A starch gel electrophoretic procedure for resolving soybean alcohol dehydrogenase band 1

Introduction: Electrophoretic analysis of plant isozyme polymorphisms has become an important tool for plant geneticists and evolutionists (Weeden and Wendel, 1989). The techniques used are dependent upon the species and enzymes studied (Kephart, 1990; Wendel and Weeden, 1989). Electrophoretic techniques for soybean include polyacrylamide gel electrophoresis (PAGE), starch gel electrophoresis, and polyacrylamide/starch gel electrophoresis (Hedges et al., in press).

Gorman and Kiang (1977) used PAGE to identify three zymogram patterns for soybean alcohol dehydrogenase (ADH, EC 1.2.3.4). Pattern 1 had a seven-band zymogram; pattern 2 had a five-band zymogram with bands 1 and 4 missing; pattern 3 had a four-band zymogram with bands 1, 4 and 5 missing. Gorman and Kiang (1978) suggested a model of inheritance for ADH bands. They postulated that bands 3, 6, and 7 are related, and that bands 1, 4 and 5 also are related; " . . . bands 1 and 5 being the respective homodimers of loci designated ADH-1 and ADH-4 and band 4 being the heterodimer formed by the combination of monomers from ADH-1 and ADH-4." The gene symbol later was changed to Adh1. Kiang and Chiang (1987), using polyacrylamide/starch gel electrophoresis, reported genetic linkage of the Adh1 locus and the W1 locus in Linkage Group 8.

Rennie et al. (1989) used starch gel electrophoresis for soybean ADH and found only two distinct invariant bands. In a 1989 personal communication to R.G. Palmer, Charlie Pedersen described a procedure using starch gel electrophoresis that consistently differentiated between soybean ADH patterns 1 and 3 but not pattern 2; Gorman and Kiang's (1977) band 1 was not resolved. Using a morpholine-citrate buffer system (pH 8.3), Delorme and Skorupska (1992) used starch gel electrophoresis for soybean ADH. They resolved four strong bands and two weak bands. They reported that their soybean ADH banding patterns did not correlate with those reported by Gorman and Kiang (1978).

The analysis of soybean ADH using PAGE and polyacrylamide/starch electrophoresis has the advantage of resolving more bands more consistently than starch gel electrophoresis, but has the disadvantage of being more expensive and involving hazardous chemicals. It would be desirable if the safer and less expensive starch gel electrophoresis procedure could be used to reliably resolve all seven bands described by Gorman and Kiang (1977). This would be especially desirable for use in further studies of Linkage Group 8, since band 1, the band which has been difficult to resolve using starch gel electrophoresis, is the putative homodimer of the locus designated Adh1 by Gorman and Kiang (1978).

This paper describes a starch gel electrophoretic procedure which resolves all seven soybean ADH bands. The methods used are based on those described by Cardy and Beversdorf (1984) and Rennie et al. (1989). Several modifications were made at different stages of the procedure.

Sample preparation: The most significant modification is to sample the cotyledons 6 h to 24 h after planting on germination paper. This is far earlier than the 96 h to 240 h recommended by Rennie et al. (1989). The intensity of band 1 is greatest when cotyledons are sampled at 6 h and is only slightly less intense when sampled at 12 h, 18 h, or 24 h. When cotyledons are sampled at 30 h, resolution of band 1 is not reliable; band 1 does not resolve when cotyledons are sampled at 36 h, 42 h, or 48 h. The seed coats are removed before sampling; this is facilitated by wetting the seed coats with distilled water. A core sample is taken through both cotyledons. Care is taken to not break off either cotyledon or damage the embryo. The young seedlings are easily transplanted to peat pots.

Samples are placed in microcentrifuge tubes with 30 uL of homogenization buffer, (16.7 g sucrose and 8.3 g sodium ascorbate added to 100 mL distilled deionized water). Tubes with reusable lids are useful if the sample is processed in stages or has to be re-run. To maintain high enzyme quality, samples are stored in an ultra-low freezer at -86°C.

Samples are ground for 45 seconds with the sample tube immersed in a small jar of ice. The sample is centrifuged for 1.5 minutes at 12,400 rpm in a Fisher Microcentrifuge, model 235B. A pre-punched wick (Northfork Products, P.O. Box 4347, Tumwater, WA 98501 USA) is placed into each sample to absorb supernatant before re-freezing.

Buffer and gel preparation: Decreasing the amount of citric acid in the electrode buffer from 1.5 g/L to 0.55 g/L increases the pH from 6.5 to 6.8 and increases separation and resolution of ADH. Buffer freshness is extremely important; use of week-old buffer results in poor resolution of band 1.

Gels are prepared with 500 mL of gel buffer (1 part electrode buffer: 3 parts distilled deionized water) and either 65 g (13% w/v), 60 g (12% w/v) or 55 g (11% w/v) of starch. Higher starch concentration improves band clarity of the stained gel slices, which is desirable if the slices are to be photographed. The starch is suspended in 150 mL of gel buffer; 350 mL of gel buffer is cooked in a microwave on high for 220 seconds before adding to the starch suspension and cooking on high for approximately 40 to 45 seconds. After being wrapped in Saran Wrap and cooled to room temperature overnight, the gels are refrigerated approximately 15 minutes before loading with samples.

Electrophoresis and staining: Gels are run 5.5 h. A pair of gels usually is run using one power source at 16 W constant wattage; a single gel is run using a power source at 8 W. After electrophoresis, a double-thickness bottom slice is used for staining to ease handling and to increase the intensity of band 1. The staining solution is a modification of that in Rennie et al. (1989); 0.15 g agar is dissolved in 10 mL 0.1 M Tris-HCl (pH 8.0) in a microwave oven on high 35 seconds to 40 seconds then added to a premixed solution of 10 mL 0.1 M Tris-HCl (pH 8.0), 2 mL (25 mg/50 mL) NAD, 2 mL (25 mg/50 mL) NBT, 0.5 mL (10 mg/50 mL) PMS, and 5 drops of 95% ethanol. The mixture is poured over the gel slice and allowed to set before incubating in the dark at 38°C. While bands 2 through 7 appear within 2 h, band 1 may not

appear for 6 h, so scoring is done the next morning.

Evaluation of banding patterns: Eight cultivars have been evaluated using this procedure: Beeson, BSR 101, Cayuga, Cutler, Jefferson, Lincoln, Swift, and Wye. We observed three banding patterns, similar to those illustrated by Gorman and Kiang (1977), among the eight cultivars. A seven-band pattern is observed for Beeson, BSR 101, Cutler and Swift. A five-band pattern is observed for Lincoln and Wye. A four-band pattern is observed for Cayuga and Jefferson (Figure 1).

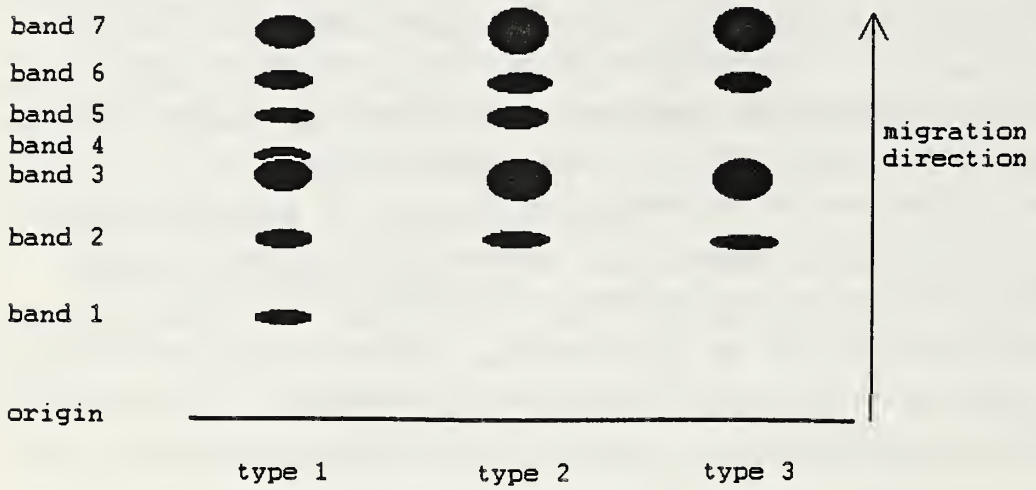


Figure 1. Observed ADH banding patterns in soybean.

There is some unexplained non-genetic variability between runs; band 1 and band 4 appear in different positions relative to the other bands. Relative position differences are associated with the citric acid content of the electrode buffer, and the wattage used during electrophoresis. Use of higher citric acid content and higher wattage results in band 1 being closer to band 2 and in band 4 being closer to band 5.

Discussion: The observed banding pattern types for Beeson, Cayuga, Cutler, Jefferson and Lincoln are the same as those reported by Gorman et al. (1982), while the banding pattern types observed for Swift and Wye are different than reported by Gorman et al. (1982). They reported a type 2 banding pattern (five bands) for Swift and a type 3 banding pattern (four bands) for Wye.

The banding patterns observed using this procedure and those described and illustrated by Gorman and Kiang (1977) are similar enough to allow the use of this safer, less expensive procedure to differentiate between soybean ADH patterns 1, 2 and 3, and to score for Adh1 in future genetic linkage studies with Linkage Group 8. However, the unexplained non-genetic variability between runs should be investigated. Human ADH is a dimeric zinc metalloenzyme (Jornvall et al. 1987). It is possible soybean ADH also may be a metalloenzyme. Citric acid, a component of the electrode buffer, is a strong chelator of divalent metal ions. The citric acid could interact with a divalent ion associated with the enzyme and alter its electrophoretic mobility. Further study of soybean ADH structure and inheritance is required.

References

Cardy, B.J. and W.D. Beversdorf. 1984. A procedure for the starch gel electrophoretic detection of isozymes in soybean [Glycine max (L.) Merr.] Dep. Crop Sci. Tech Bull. 119/8401. Univ. of Guelph, Ontario, Canada.

Delorme, R.M. and H. Skorupska. 1992. New isoenzymes systems in soybeans. Soybean Genet. Newsl. 19:169-173

Gorman, M.B. and Y.T. Kiang. 1977. Variety specific electrophoretic variants of four soybean enzymes. Crop Sci. 17:963-965.

Gorman, M.B. and Y.T. Kiang. 1978. Models for the inheritance of several variant soybean electrophoretic zymograms. J. Hered. 69:255-258.

Gorman, M.B., Y.T. Kiang, Y.C. Chiang and R.G. Palmer. 1982. Electrophoretic classification of the early maturity groups of named soybean cultivars. Soybean Genet. Newsl. 10:143-156.

Hedges, B.R., R.G. Palmer and L.A. Amberger. nd. Electrophoretic analyses of soybean seed proteins. In: H.F. Linskens and J.F. Jackson (eds.), Modern Methods of Plant Analysis, Springer-Verlag Publishers, New York, in press.

Jornvall, H., J. Hempel and B. Vallee. 1987. Structures of human alcohol and aldehyde dehydrogenases. Enzymes 37:5-18.

Kephart, S.R. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. Amer. J. Bot. 77:693-712.

Kiang, Y.T. and Y.C. Chiang. 1987. Genetic linkage of the Adh1 and W1 loci in soybeans. Genome 29:582-583.

Rennie, B.D., M.L. Thorpe and W.D. Beversdorf. 1989. A procedural manual for the detection and identification of soybean [Glycine max (L.) Merr.] isozymes using the starch gel electrophoretic system. Dep. Crop Sci. Tech. Bull. Univ. of Guelph, Ontario, Canada.

Weeden, N.F. and J.F. Wendel. 1989. Genetics of plant isozymes. In: D.E. Soltis and P.S. Soltis (eds.), Isozymes in Plant Biology, pp. 46-72. Dioscorides Press, Portland, Oregon.

Wendel, J.F. and N.F. Weeden. 1989. Visualization and interpretation of plant isozymes. In D.E. Soltis (eds.), Isozymes in Plant Biology, pp. 5-45. Dioscorides Press, Portland, Oregon.

K.S. Haack
T.D. Couch USDA
J. Imsande
R.G. Palmer USDA

Department of Biology
NORTHERN ARIZONA UNIVERSITY
Flagstaff, AZ 86011-5640
and
USA-ARS
IOWA STATE UNIVERSITY
Ames, IA 50011

1) RFLP fragments assigned to particular genotypes

Frequently when RFLP germplasm surveys are published, the raw data matrix associated with the analysis is omitted. The data matrices can be rather large and many journals refuse to include them in referred articles. However, for researchers involved with such studies, these can be very valuable. In the future, the Soybean Genetic Database will act as repository for such data. Meanwhile, the Soybean Genetics Newsletter can serve as a medium for dissemination of such data. Below is the data matrix associated with analyses reported by Keim, Shoemaker and Palmer in Theoretical and Applied Genetics 77:786-792. 1989.

Paul Keim
Randy Shoemaker

Key to RFLP Fragments in the Data Matrix

Column	Probe	Enzyme	Fragment(Kb)
1	NE-10	EcoR1	10
2	Null allele for 1		--
3	NE-10	EcoR1	5
4	Null allele for 3		--
5	pK-3	HinDIII	7
6	pK-3	HindIII	6
7	M224	EcoR1	3.5
8	Null allele for 7		--
9	M224	EcoR1	1.3
10	Null allele for 9		--
11	M121	BclI	6.7
12	M121	BclI	4.7
13	M109	BclI	5.5
14	M109	BclI	2.3
15	M109	BclI	0.9
16	pG-15	EcoRI	8.4
17	pG-15	EcoRI	4.2
18	pG-15	EcoRI	3.5
19	pG-17.3	EcoR1	9.6
20	pG-17.3	EcoRI	6.6
21	pG-17.3	EcoRI	4.4
22	pG-17.3	EcoRI	3.8
23	pG-17.3	HinDIII	3.0
24	null allele for 23		--
25	M69	DraI	4.3
26	null allele for 25		--
27	M69	DraI	2.5
28	null allele for 27		--
29	M69	DraI	2.0
30	M69	DraI	1.9
31	M373	EcoRI	4.2
32	M373	EcoRI	3.8
33	pGly-3	HinDIII	4.4
34	pGly-3	HinDIII	3.9
35	pG21-3	DraI	1.5
36	pG21-3	DraI	8.8

Germplasm	Fragment alleles																																		
Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
PI 424.004	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	1
PI 468.904	1	0	0	1	0	1	1	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	1
PI 468.905	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1
PI 468.906	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	1
PI 468.918	1	0	1	0	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	1
PI 342.618	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1
PI 326.581	1	0	1	0	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	1
PI 153.292	1	0	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1
PI 79.593	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1
PI 408.272	0	1	0	1	0	1	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1
PI 398.303	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1
PI 407.900	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	0	1	0	1	0	0	1	1	0	0	1	1	0	0	1	0	1	0	1
Aoda	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1
Minsoy	1	0	1	0	0	1	1	0	0	1	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0
Noir	1	0	0	1	1	0	1	0	0	1	1	0	0	0	1	0	1	0	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1
T248	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	0	1	0	0	1	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1
PI 437.477	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1
PI 91.732	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
Peking	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	0	1	0	0	1	1	0	0	1	1	0	1	0	0	1	1	0	0	1
Illini	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	1	0	1	0	0	1
Asgrow X25AF	1	0	0	1	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1
Will.'82	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
Harper	0	1	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
A80-244036	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	1	0	0	1
PI 468.916	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	1
BSR-101	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
Pucker-1	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
PI 54.610	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1
Mandarin**	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1
PI 240.664	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	1	0	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1
Hodgson'78	1	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1
Dunfield	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	1	0	0	1
Richland	0	1	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1
Wilson	0	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1
CNS	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	1	0	0	1
Palmetto	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	0	1
Seneca	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	1	0	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1
Tokyo	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1
A.K. Harrow	0	1	0	1	1	0	1	0	1	0	1	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	1	0	1	0	0	1
Polysoy	0	1	0	1	1	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1
S100	0	1	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
Biloxi	0	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	1	0	0	1
Manchu	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	0	1	0	1	1	0	0	1
Hark	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1
Corsoy	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1
Harosoy	0	1	1	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
Mukden	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	1
Altona	1	0	0	1	1	0	1	0	1	0	0	1	1	0	1	1	0	0	1	0	0	1	1	0	1	0	0	1	1	0	0	1	1	0	0	1
Roanoke	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	1
Maple Arrow	1	0	0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1
Jilin 3	1	0	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0	1	0	1
Medium Green	1	0	0	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	0	1	0	0	1	1	0	1	0

*Genotypes 'Stine 3200', 'Trivalley Charger', 'A81-356022' and 'MidWest Oil Seeds 3010', 'Pride B216' had identical scores with Williams'82 and are not included here. **Genotypes Mandarin and Mandarin (Ott.) were identical in this study.

USDA/ARS
Iowa State University
Ames IA 50011

1) A rapid method of regeneration and *Agrobacterium*-mediated transformation of soybean:

In recent years there have been numerous reports of regenerating plants from tissue explants, cultured cells and protoplasts of many species; however, the soybean has not proven generally responsive to procedures that have been successfully applied to other species. Successful application of standard genetic engineering procedures to the soybean has been limited by the lack of an efficient transformation and regeneration system.

Various tissue explants have been tested as sources of multiple shooting, but induction of adventitious budding and subsequent plant formation has been restricted to seedling tissue such as cotyledonary axillary bud explant (Evans, 1981), cotyledonary node segments (Cheng et al., 1980), and hypocotyl sections (Kimball and Bingham, 1973).

Several groups have recently developed methods for transformation and regeneration of soybean tissue (Brawale et al., 1986; Lazzeri et al., 1985; Wei and Xu, 1988). Newell and Luu (1985) reported the isolation of protoplasts from seedling hypocotyls of *Glycine canescens* that later developed to form multicellular colonies, calli, and shoots. The frequency of regeneration was low but reproducible. A regeneration procedure by organogenesis in *Glycine max* was described by Wright et al., (1986). They were able to obtain viable soybean plants from calli derived from immature embryos. The regeneration which occured via somatic embryogenesis or organogenesis was successful in the production of viable, fertile plants in 54 soybean genotypes. Wright et al., (1987) reported a successful technique for the initiation and proliferation of shoot epicotyl tissue of soybean. Fertile plants were recovered from shoots developed from epicotyl-derived callus.

Finer and Nagasawa (1988) described a method for the initiation of somatic embryos from immature zygotic embryos of soybean. Somatic embryos were observed 4 weeks after cultures were initiated. Secondary

somatic embryos were proliferated, following transfer, from the apical or terminal portions of the older primary somatic embryos.

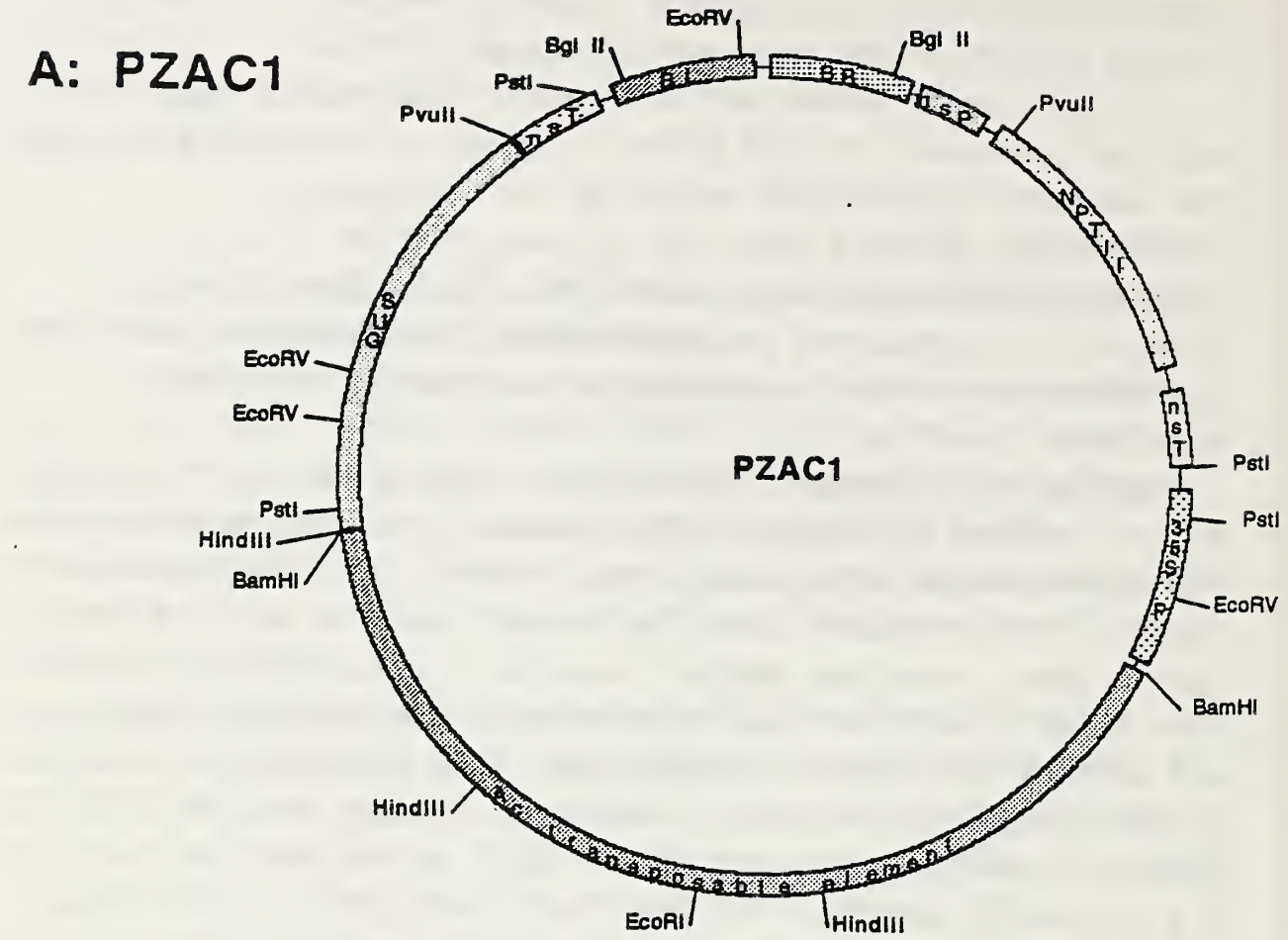
Most of these methods require extensive tissue culture steps which significantly increase the time period to recover a mature soybean plant. The regeneration percentages as well as the percentage of transformations obtained from most of these methods were very low (Chee et al., 1989; and McCabe et al., 1988). We report here a rapid procedure for regeneration and transformation of soybean cotyledons by *Agrobacterium*-mediated transformation and recovery of stabley transformed plantlets.

Materials and Methods: Seeds of *Glycine max* L. Merr. cv 'Peking' were surface sterilized by soaking in 70% ethanol, for 2 minutes, rinsed twice with sterile distilled water, then soaked in 20% sodium hypochloride solution for 15 minutes followed by several rinses in sterile distilled water. Seeds were then imbibed overnight in sterile distilled water at room temperature. Seed coats were removed, cotyledons were separated and embryos were removed and discarded. The half-cotyledons were then placed in modified MS medium (Murashige and Skoog, 1962) which contains 5mg/L of 6-benzyl-amino purine (BAP), 15g/L sucrose pH6.5 for 24 hours. The cotyledons were then inoculated with overnight cultures of virulent *Agrobacterium* strain A281 containing the binary vectors PZAC1, PZAC1/R (Fig. 1A,1B), and PZA3 (Fig. 1C) (Zhou and Atherly, 1989).

Inoculation was accomplished by inducing wounds at the proximal end of the meristematic region of embryo attachment to the cotyledons with a sterile needle. The cotyledons were transferred into 1.5 inch Petri dishes containing 10 ml of YEP culture, (16g/L Bacto-peptone, 16g/L sodium chloride, and 8g/L yeast extract, pH 7.5) cultured with *Agrobacterium*, and incubated for 15 minutes at room temperature.

Fig. 1: The circular and the linear maps of *Agrobacterium tumefaciens* vectors used for the inoculation of soybean tissue (redrawn from Zhou and Atherly, 1989).

A: Circular restriction of PZAC1 vector (the region between the left and the right border (7.7kb) is not included in this figure so other fragments can be magnified.
B: The linear map of PZAC1/R vector.
C: The linear map of PZA3 vector.


A: PZAC1
EcoRV
Bgl II
Bgl II
PstI
PvuII
PvuII
EcoRV
EcoRV
PstI
HindIII
BamHI
PstI
PstI
EcoRV
BamHI
HindIII
EcoRI
HindIII
PZAC1
B: PZAC1/R
RB
NPT II
35S p
Ac
GUS
nos p
nos t
nos t
C: PZA3
RB
NPT II
35S p
GUS
LB
nos p
nos t
nos t

The cotyledons were blotted on sterile filter paper to remove the excess liquid, then transferred to MS-BAP plates for a further 48 hours incubation. Cotyledons were then transferred to shooting medium.

The shooting-induction medium consisted of a modified Murashige and Skoog (1962) basal medium, to which was added 1mg/L thiamine-HCl, 0.5 mg/L pyridoxine-HCl, 2X MS micronutrients salts (including Fe), 2X MS macronutrients salts, B5 vitamins (Gamborg et al., 1968), 25 g/L sucrose, 7 g/L Difco Bacto agar, pH 7.0. All ingredients were added before autoclaving for 16 minutes at 121C. After autoclaving the medium was cooled to approximately 50C and 10 mg/L Carbenicillen and 250 mg/L Cefotaxime (Sigma) were added, mixed, and then poured into Petri dishes.

After 7 days cotyledons were transferred to a selection medium. The selection medium was the shooting-induction medium plus 200 mg/L Kanamycin. The cotyledons were transferred each 2 weeks to fresh selection medium until the explants were ready to transfer to rooting medium. The rooting medium` contained MS macronutrient salts, MS micronutrient salts, B5 vitamins, 10g/L sucrose, 2g/L Gel-rite, and 300mg/L Cefotaxime pH5.8. The medium was poured into Magenta boxes (Magenta Corporation) with approximately 50 ml each. Multiple shoots from each cotyledon were excised individually by dissecting with a sterile surgical knife after they reached 1 cm in length and then transferred to the rooting medium. Shoots were transferred to fresh boxes every 14 days until they formed good root systems. Regenerated plantlets with healthy roots were planted in sterile vermiculite for 7-14 day. Plantlets were then hardened-off and transferred to the greenhouse. Seeds were harvested from R0 plants and replanted in pots in the greenhouse to obtain R1 plants.

Results and Discussion Soybean plant regeneration and transformation. The number of plants regenerated from soybean cotyledons is presented in Table 1. The number of shoots regenerated per cotyledon differed from one cotyledon to another, and ranged from 2-15. Cotyledons that regenerated only one shoot were discarded. The total number of plants that survived several cycles of Kanamycin selection was 331 (Table 1). Many shoots could not survive the first cycle of selection, suggesting that those shoots were not transformed with vectors

containing the Kanamycin resistance gene.

PCR analysis was conducted on surviving RO plants using oligonucleotide primers that delineated a specific sequence located in Ac. A transformation percentage of 24.31 and 10.48 (the percentage of plants containing the Ac element from the total survived plants) were obtained when plasmids PZAC1 and PZAC1/R were used respectively (Table 1). As far as we aware the inoculation procedure used in this study was more efficient than other reported transformation procedures. Using the plasmid PZAC1/R, which lacks the left-border sequence of the T-DNA, resulted in the lower transformation percentage of 10.48 but this frequency is higher than other transformation procedures (Chee et al., 1989, Hinchee et al., 1988, and McCabe et al., 1988). This regeneration and transformation procedure can produce mature soybean plants in a period of 12-14 weeks.

The selection system we employed using 200mg/L Kanamycin enabled us to detect the probable presence of the NPTII gene in transformed tissue. Inoculated cotyledons were cultured on fresh media containing 200mg/L of Kanamycin every 14 days. This provided a continual supply of fresh Kanamycin to minimize the probability of antibiotic depletion and subsequent escapes. Untransformed cells died in the beginning of the first cycle of selection. The early signs of dying tissue started first, in and around the inoculated area, and then spread to the whole cotyledon. The survival of shoots that arose from cotyledons after several Kanamycin selection cycles suggested that those plants were regenerated from transformed tissue. The percentage of regenerated plants from the total number of inoculated cotyledons was 14.8 and 7.2 when PZAC1 and PZAC1/R were used for the inoculation.

All regenerated plants were fertile and produced seed which was then replanted in pots in the greenhouse to obtain R1 plants. Further studies will be done with the goal to use this system for Ac tagging and clonning of soybean genes.Table 1: Number of surviving transgenic plants regenerated from cotyledons inoculated with *Agrobacterium tumefaciens* containing plasmids PZAC1, pZAC1/R, and PZA3.

	# of cotyledons inoculated	# of regenerated plants	% of regeneration	# of transformants
PZACI	1227	181	14.8	44 (24%) a
PZACI/R	1156	83	7.2	9 (10.5%) b
PZA3	764	67	8.8	n.t c
TOTAL	3147	331	10.52	

a The percentage of plants containing the Ac element from the total number of regenerated plants when PZAC1 vector was used.

b The percentage of plants containing the Ac element from the total number of regenerated plants when PZAC1/R vector was used.

c n.t=not tested

REFERENCES

Barwale U.B., H.R. Kerns, J.M. Widholm.1986. Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. Planta 167:473-481.

Chee P.P., K.A. Fober, and J.L. Slightom.1989. Transformation of soybean (*Glycine max*) by infecting germinating seeds with *Agrobacterium tumefaciens*. Plant Physiol. 91:1212-1218.

Cheng T.Y., T. Saka, T.H. Voqui-Dinh.1980. Plant regeneration from soybean cotyledonary node segments in culture. Plant Sci. Lett. 19:91-99.

Evans D.A. 1981. Soybean tissue culture. Soybean Genetics Newsletter 8:27-29.

Finer J.J. and A. Nagasawa (1988): Development of an embryogenic suspension culture of soybean (*Glycine max* L.Merr.) Plant Cell Tissue and Organ Culture. 15:125-136.

Gamborg O.L., R.A. Miller, K. Ojima.1968. Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151-158.

Kimball S.L., E.T. Bingham.1973. Adventitious bud development of

soybean hypocotyl sections in culture. Crop Sci 13:758-760.

McCabe D.E., W.F. Ewain, B.J. Martinell, and P. Christou. 1988. Stable transformation of soybean Glycine max by particle acceleration. Biotechnology. 6:923-926.

Murashige T., F. Skoog.1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physio Plant 15:473-497.

Newell C.A. and H.T. Luu 1985. Protoplast culture and plant regeneration in *Glycine canescens* . Plant Cell Tissue and Organ Culture. 4:145-149.

Wei Z-M and Z-L Xu .1988. Plant regeneration from protoplasts of soybean (*Glycine max* L.). Plant Cell Reports 7:348-351.

Wright M.S., S.M. Koehler, M.A. Hinchee, and M.G. Carnes. 1986. Plant regeneration by organogenesis in *Glycine max.* Plant Cell Reports. 5:150-154.

Wright M.S., M.H. William, P.E. Pierson, and M.G. Carnes.1987. Initiation and propagation of *Glycine max* L. Merr. plant from tissue-cultured epicotyls. Plant Cell Tissue and Organ Culture. 8:83-90.

Zhou J.H., A.G. Atherly.1989. Insertion of the transposable element Ac into soybean by transformation. Soybean Genetics Newsletter 16:124-126 .

Salah Muhawish Al-Janabi
Randy C. Shoemaker

Iowa State University
USDA/ARS
Ames IA 50011

2) Rapid method for preparing double-stranded template DNA for Polymerase Chain Reaction amplification from soybean.

In the last three years PCR has become an important method for detecting and mapping specific DNA sequences in plants. For example, a rapid method for identifying DNA sequences linked to known genes, called RAPD (Random Amplification of Polymorphic DNA) is based upon a recent modification of the polymerase chain reaction (PCR) in which synthetic primers are used to amplify specific sequences from genomic DNA. This technique allows rapid identification of sequences linked to genes of interest (Martin et al. 1991). PCR can also be used to detect transgenes in putative transgenic plants.

PCR requires only a small amount of templete DNA (10pg) to amplify even single copy genes. Since hundreds of lines often need to be tested for polymorphic DNA, the need for rapid and inexpensive techniques to isolate genomic DNA has become increasingly important.

The precedures available for isolating plant genomic DNA (Saghai et al. ,1984, Kiem et al., 1988), are time consuming and expensive. They require a large amount of freeze-dried plant tissue and a minimum of 4-5 days from collecting leaves from seedlings to obtaining genomic DNA. We have developed a rapid method of extracting DNA which produce DNA within 3-4 hours from collecting leaves from soybean seedling.

We demonstrate that DNA prepared in this mannar is sufficiently pure to serve as a templete for PCR amplification by amplifying a 615bp fragment of the Ac element from DNA isolated by this method from transgenic soybean plants containing the Ac.

Materials and methods: The leaf materials used in this study were from tissue culture regenerated soybean plants. Plants were regenerated from cotyledonary node tissue that had been inoculated with *Agrobacterium tumefaciens* harboring the binary vectors PZAC1, PZAC1/R, and PZA3 (Zhou and Atherly,1989). We were aiming toward the insertion

of the Ac maize transposable element into soybean by *A. tumefaciens*-mediated transformation.

DNA was extracted from one fresh leaf (50 mg or less) of each Ro plant. 200µl of nuclei isolation buffer (20mM Pipes, pH7.0, 3mM $MgCl_2$, 0.5M Hexalyne Glycol, 10mM-Orthophenanthroline, 10mM sodium Bisulfate) was added to 1.5ml eppendorf tubes containing the leaves samples. Using a sterile pistil, leaves were ground for 30-45 seconds with slight up and down motions until evenly macerated. The tubes were then spun in a microcentrifuge for 5 seconds. 20µl of 20% Triton X-100 added and mixed by gently flicking the tubes. Next, 250µl of freshly prepared SDS-lysing buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl pH8.0, 10mM orthophenanthroline, 10mM sodium bisulfate, and 10µg/ml proteinase K) was added and mixed thoroughly by inversion. Tubes were incubated at 65C for 60 minutes, with mixing by inversion every 15-20 minutes. The tubes were then spun for 5 seconds in a microcentrifuge, followed by the addition of 200µl of 10M sodium acetate. Tubes were mixed by inversion and chilled on ice for 10 minutes. To precipitate the remaining tissue, tubes were spun for 5 minutes in a micocentrifuge.

The supernatant was transferred to fresh tubes, and extracted two times with phenol:chloroform:isoamyl alcohol (25:24:1). The DNA was precipitated by adding a half volume of isopropanol, incubating 1 hour at -20C and spinning 10 minutes in a micocentrifuge. The DNA pellet was washed in 70% ethanol, dried, and resuspended in 30µl of sterile distilled water.

Results and discussion: The amount of DNA extracted from one leaf by this method was approximately 1-5µg, which is sufficient to run hundreds of PCR reactions. To confirm the suitability of the isolated DNA for PCR amplification, PCR analysis was used to amplify a specific DNA fragment of the Ac element inserted in the transgenic soybean plants. Two specific oligonucleotide complementary to the Ac element were synthesized. The oligonucleotide sequences were selected from the 1.6kb HindIII fragment within the Ac sequence. The first primer (23 nucleotides) started at the nucleotide 1857, and the second (24 nucleotides) begun at nucleotide 2448, 615bp down stream the Ac element sequence. The two oligomers were tested by amplifying the 615bp sequence within the Ac element by using 10ng of the plasmids PZAC1,

PZAC1/R, PZA3,and soybean genomic DNA using conditions described in materials and methods. PZA3 and untransformed soybean DNA were used as negative control, while PZAC1 and transformed tobacco DNA served as a positive control templates. PCR amplification cycles were controlled by Coy-Temp-Cycler (Coy laboratory Products Inc.). The following cycles were used; denaturation of duplex DNA at 93C for 2 minutes, followed by primer annealing at 60C for 45 seconds and extension at 72C for 2.5 minutes. The amplifications were carried out for 40 cycles. Amplification of the 615bp fragment was detected by agarose gel electropheresis and rechecked by Southern blot (Southern 1975) with [32P]-dCTP-labeled 1.6kb HindIII internal fragment of the Ac element that encompasses the described 615bp fragment .

Ethidium bromide gel and southern blot analysis of genomic DNA isolated from transformed plants indicated the presence of the amplified 615bp fragment in transformed soybean plants. Therefore, the genomic DNA extracted by this method is of high quality enough to serve as template for PCR amplification.

This method of DNA extraction can have a great impact on identifying known loci or on mapping plant genomes when random amplification of polymorphic DNA (RAPD) is used since it reduces the time required for DNA extraction from plant materials. This allows mapping of large number of loci in a relatively short time as compared to RFLP mapping which requires more time for DNA preparation and Southern hybridization. It can be used to analyze a segregating population for nonmorphological traits. This can be done by extracting DNA from individual plants and testing it with RAPD-PCR technique to amplify the polymorphic fragments tagging the gene or trait and selecting accordingly.

References:

Keim P, T.C. Olson , R.C. Shoemaker.1988. A rapid protocol for isolating soybean DNA. Soybean Genet. Newsl.15:150-152.

Martin G.B., J.G. Williams, and S.D. Tanksley.1991. Rapid identification of markers linked to a pseudomonas resistance gene in tomato using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. USA 88:2336-2340.

Saghai-Maroof M.A., K.M. Soliman, R.A. Jorgensen, R.W. Allard . 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA **81**:8014-8018.

Southern EM.1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503-517.

Zhou JH, Atherly AG (1989). Insertion of the transposable element Ac into soybean by transformation. Soybean Genet. Newsl.16:124-126.

Salah Muhawish Al-Janabi
Randy C. Shoemaker
USDA-ARS-FCR

IOWA STATE UNIVERSITY
Department of Genetics, Agronomy and Botany
Ames, Iowa 50011
and
NORTH CAROLINA STATE UNIVERSITY
Raleigh, NC 27650
and
United States Department of Agriculture

1) Genetic studies of a male-sterile, female-sterile mutant from a transposon-containing soybean populations

An unstable mutation for anthocyanin pigmentation in soybean was identified in 1983. The mutability is conditioned by an allele at the w4 locus that is recessive to wild type. This is the w4-m line and it was assigned Genetic Type Collection Number T322.

In a transposon tagging study, we screened progenies of germinal revertant at the w4-m locus for new mutations (Palmer et al., 1989). One of the new mutations, recovered as a single plant, was almost completely sterile except for a few pods.

Our objectives were: 1) to determine the inheritance; 2) to determine allelism with known male-sterile, female-sterile mutants, st2, st3, st4, and st5; and 3) to check linkage with w4, five chlorophyll-deficient mutants, and three necrotic root mutants. Experiments are continuing with the st3 mutant and the three necrotic root mutants and will not be reported in this Newsletter article.

Materials and methods: The inheritance of an 'unknown sterile', st st, was obtained from our allelism study and from our linkage study. Allelism tests were conducted with four nonallelic mutants, St2 st2 (T241H), St4 st4 (T258H), and St5 st5 (T271H). The sterile mutant designated st1 is no longer available. F_1 and F_2 populations of each cross were observed for fertile and sterile plants. If two lines were allelic with regard to their sterility, then one out of four F_1 plants would

be sterile; in the F_2 generation, nonsegregating families and families segregating 3 fertile : 1 sterile plants would be observed. If different genes were controlling sterility in the two lines, no sterile plants would be observed in the F_1 generation. Moreover, the F_2 generation would include nonsegregating families, families segregation 3 fertile : 1 sterile plants, and families segregating 9 fertile : 7 sterile plants.

The Harosoy near-isogenic w4 line was used in the linkage test with the unknown sterile mutant. Four chlorophyll-deficient mutants derived from the transposon tagging study was used. The T323, T324, and T325 mutants have different chlorophyll-deficient phenotypes and are malate dehydrogenase null for one of the malate dehydrogenase enzymes, yet all three mutants are allelic. The fourth chlorophyll-deficient mutant was an unknown yellow designated RT-753. With the T323, T324, T325, and RT-753 mutants, it was difficult to classify accurately the yellow plant for fertility or sterility because the plants were weak. Fertile green F_2 plants were single-plant threshed and evaluated as F_3 plants in F_2 plant-progeny rows. The fifth chlorophyll-deficient mutant, CD-5, (Y y) has a yellow-green phenotype and upon self pollination gives 1 green : 2 yellow-green : 1 yellow lethal seedling.

Results and discussion: Inheritance of the 'unknown sterile' Single-plant progeny rows of F_2 families were classified as either segregating for sterility or not segregating. In segregation families of St st with homozygous fertile plants, we observed a ratio of 3 fertile : 1 sterile plants (Table 1). Chi-square tests for homogeneity showed that all progenies were drawn from the same population (data not presented). These results are expected if sterility was caused by a single recessive gene.

Allelism test with the 'unknown sterile' No sterile plants were found in 57 F_1 plants obtained from the cross St2 st2 x St st, in 44 F_1 plants obtained from the cross St4 st4 x St st, or in 10 F_1 plants obtained from the cross St5 st5 x St st. F_2 seed were harvested from the F_1. The F_2 populations were divided into

three classes; nonsegregating, segregating 3 fertile : 1 sterile plants, and segregating 9 fertile : 7 sterile plants. The data fit the expected 1:2:1 ratio (Table 2).

The segregating F_2 populations were divided into two groups on the basis of chi-square values (Table 3). One group represented a 3:1 population; the other group a 9:7 population. Chi-square tests for homogeneity for the various allelism tests showed that progenies were drawn from the same population (Table 3). These results agree with the hypothesis that the recessive allele in St st is different from the alleles in T241H, T258H, and T271H. We need to complete the allelism test with T242H(St3 st3) before a Genetic Type Collection Number and gene symbol can be assigned to the 'unknown sterile'.

Linkage test with the 'unknown sterile' Recombination percentages for independent assortment between st and near-white flower, w4, and chlorophyll-deficient, y, (CD-5) were calculated from F_2 data. The segregation data for purple : near-white flower and fertile : sterile plants fit the expected 3:1 ratio (data not presented). Chi-square tests for homogeneity showed that all progenies were drawn from the same population (Table 4). No linkage was detected between st and w4 (Table 4).

The segregation data for CD-5 gave 1 green: 2 yellow-green: 1 yellow lethal which fit the expected ratio and the data for 3 fertile: 1 sterile plants also fit the expected ratio (data not presented). No chi-square tests for homogeneity for the F_2 families were performed. The number of F_2 seed per F_1 plant was small because the yellow-green F_1 plants were weak and produced only 10-30 seed. A total of 56 yellow-green F_1 plants were harvested and then progeny evaluated. No linkage was detected between y and st (Table 5).

Fertile green F_2 plants from the unknown sterile with T323, T324, T325, and RT-753 were single plant threshed. These progenies were evaluted as F_3 families. No linkage was detected between st and any of the four chlorophyll-deficient mutants (Table 6).

Summary: The 'unknown sterile' is a single-gene recessive and is male-sterile and almost completely female-sterile. The sterile mutant is non-allelic to st2, st4, and st5. The sterile mutant is not linked to flower color mutant w4, or to chlorophyll-deficient mutants CD-5, T323, T324, T325 and RT-753. Genetic tests with st3 and the three necrotic root mutants are not yet completed.

R.G. Palmer-USDA
Zhang Fan
H.T. Horner

REFERENCES

Palmer, R.G., B.R. Hedges, R.S. Benavente, and R.W. Groose. 1989. w4-mutable line of soybean. Dev. Genet. 10:542-551.

Table 1. Segregation for fertile and sterile soybean plants in F_2 families from crosses of heterozygous 'unknown sterile' plants St st with homozygous plants St2 St2, St4 St4, and St5 St5.

Cross combination	No. F_2 Plants		X^2	
	Sterile	Fertile	(3:1)	P
St2 St2 x St st	1239	398	0.41	0.52
St4 St4 x St st	927	301	0.16	0.69
St5 St5 x St st	415	146	0.31	0.58

Table 2. Segregation for fertile and sterile soybean plants in the F_1 generation and in F_2 families from crosses of heterozygous 'unknown sterile' plants St st with heterozygous plants St2 st2, St4 st4, St5 st5.

Cross combination	F_1 plants		No. F_2 families			X^2	
	No.	Phenotype	NS†	3:1	9:7	(1:2:1)	P
St2 st2 (T241H) x St st	57	Fertile	12	29	16	0.58	0.75
St4 st4 (T258H) x St st	44	Fertile	10	19	15	1.95	0.38
St5 st5 (T271H) x St st	10	Fertile	3	3	4	‡	

† NS=non-segregating sterile plants
‡ Not tested because too few families

Table 4. Linkage test of the 'unknown sterile' phenotype with the w4 locus in F_2 soybean populations

Cross combination	No. of F_2 plants						
Harosoy w4 w4 St St x W4 W4 St st	W4 __ St __	w4 w4 St __	W4 __ st st	w4 w4 st st	df	X^2 (9:3:3:1)	P
Total	582	182	182	80	12	28.19	0.01
Pooled					1	5.09	0.02
Homogeneity					11	23.10	0.00

Table 5. Linkage test of the 'unknown sterile' phenotype with the CD-5 (Y y) locus in F_2 soybean populations

Cross combination	No. of F_2 plants					X^2	
	St __ Y Y	St __ Y y	st st Y Y	st st Y y	y y*	(3:6:1:2:4)	P
Y y St St x Y Y St st	209	404	75	142	244	3.90	0.42

*This genotype (y y) is a seedling lethal.

Table 3. Segregation for fertile and sterile soybean plants in F_2 families from crosses of heterozygous 'unknown sterile' plants St st with homozyous plants St2 st2, St4 st4, and St5 st5.

Cross combination		No. of F_2 plants			X^2		No. of F_2 plants			X^2	
		Fertile	Sterile	df	(3:1)	P	Fertile	Sterile	df	(9:7)	P
St2 st2 (T241H) x St st											
	Total	3857	1282	29	18.30	0.94	1588	1240	16	3.16	0.99
	Pooled			1	0.01	0.93			1	0.01	0.92
	Homogeneity			28	18.29	0.92			15	3.15	0.99
St4 st4 (T258H) x St st											
	Total	3338	1109	19	4.68	0.99	2094	1639	15	6.90	0.96
	Pooled			1	0.01	0.92			1	0.04	0.85
	Homogeneity			18	4.67	0.99			14	6.87	0.94
St5 st5 (T271H) x St st											
	Total	278	84	3	0.84	0.84	324	269	4	1.52	0.82
	Pooled			1	0.62	0.43			1	0.63	0.43
	Homogeneity			2	0.22	0.89			3	0.89	0.83

Table 6. Linkage tests of the 'unknown sterile' phenotype with chlorophyll-deficient (CD) plants of T323, T324, T325 and RT-753. Fertile green foliage F_2 plants, from F_2 families segregating for sterility and chlorophyll deficiency, were single-plant threshed and evaluated in the F_3.

	No. of F_3 families					
	Non-segregating sterile		Segregating sterile		X^2	
Cross combination	Nonseg. CD	Seg. CD	Nonseg. CD	Seg. CD	(1:2:2:4)	P
St st x T323	23	41	38	66	2.38	0.50
St st x T324	11	27	25	59	1.07	0.78
St st x T325	10	15	22	38	1.35	0.72
St st x RT-753	27	56	52	129	2.22	0.53

PIONEER HI-BRED INTERNATIONAL
Johnston, IA
and
SOYBEAN WINTER NURSERY
Los Andes, Chile

1) Identification of restriction fragment length polymorphisms (RFLP's) to map soybean cyst nematode resistance genes in soybean

Introduction: The soybean cyst nematode, SCN, (Heterodera glycines Ichinohe) is an economically significant pest of soybean in the USA. It has been reported in nearly all states in which soybean is grown. Despite its importance, the genetics of host resistance in the SCN-soybean system are poorly understood. Definitive genetic studies require the development of genetically defined hosts and parasites. Restriction fragment length polymorphisms (RFLP's) can be utilized to characterize soybean genotypes segregating for resistance to the SCN and potentially locate regions of the soybean genome where these genes governing resistance to SCN reside. Mapping genes for SCN resistance requires a soybean population segregating for RFLP's throughout the genome and for resistance to SCN. A soybean population composed of 330 $F_{2:7}$ near-recombinant inbred lines (RIL) was developed at Iowa State University to map genes conditioning resistance to the SCN (L.Mansur, unpublished data). The population was developed by inbreeding by single seed descent an F_2 population obtained from the cross PI 437654 x BSR 101. PI 437654 is unique among SCN-resistant soybean genotypes because it has been reported to be resistant to all the known races of SCN in the USA. (Myers and Anand, 1991). We report here our preliminary observations on the DNA polymorphisms detected between PI 437654 and BSR 101. These polymorphisms can be used in co-segregation analysis to locate SCN-resistance genes found in PI 437654 as well as other important traits segregating in this cross (i.e., brown stem rot (Phialophora gregata) resistance found in BSR 101).

Methods: Total DNA was isolated from the leaves of the soybean cultivars PI 437654 and BSR 101 as previously described

(Keim et al., 1988). Ten micrograms of PI 437654 and BSR 101 DNA were digested to completion with 10 to 15 units of the enzymes Eco RI, Hind III, Bgl II, Eco RV, Dra I, Taq I, and Xba I. After digestion, restricted DNA fragments were separated in 0.8% agarose (BRL #5510 UB) by electrophoresis. Separated fragments were then transferred onto Zeta-Probe nylon membrane (BRL). Plasmids containing random cloned soybean fragments available from public genomic libraries (Apuya, et al. 1988; Keim and Shoemaker, 1988) were cut with Pst I restriction enzyme and the insert was separated from the vector in low melting point agarose (BRL #5517) by electrophoresis. The inserts were cut out of the gel and labeled with 32-P by random priming. Labeled probes were used in hybridization experiments with membranes containing total DNA digests of PI 437654 and BSR 101 with the 7 enzymes described above. For segregation analysis, 120 recombinant inbred line DNAs were digested with Eco RI, transferred onto nylon membranes and probed with a random probe that had detected polymorphisms between PI 437654 and BSR 101.

Results and discussion: We have initiated a project to map genes governing resistance to different races of the soybean cyst nematode. Previous RFLP analyses (Apuya et al., 1988: Keim et al., 1989) of soybean have had relatively few polymorphisms detected within Glycine max. In contrast, our hybridization experiments between labeled probes and restriction-digested DNA of PI 437654 and BSR 101 indicated that a high level of polymorphisms exists between these two Glycine max genotypes (Table 1). The restriction enzyme Hind III detected the most polymorphisms with 41%, while the least informative was Bgl II with 17%. Eco RI, Dra I, Taq I, and Eco RV were almost equally valuable in detecting polymorphisms with 33%, 31%, 30%, and 29%, respectively. These results indicated that Hind III and Eco RI, the least expensive restriction enzymes, could be preferentially used for segregation analysis in this cross, minimizing the costs of the mapping effort. A set of 120 near-RIL DNAs was digested with Eco RI and hybridized with a polymorphic probe designated as pB-032 (Keim and Shoemaker, 1988). The segregation for this RFLP

among the 120 near-RILs was as expected, not significantly different ($P<0.05$) from the ratio of 1:1. Assuming segregation for SCN resistance, the normal segregation of this marker indicated the potential of this RIL population from PI 437654 x BSR 101 to map genes governing resistance to SCN.

Table 1. Percentage of probes detecting polymorphisms with a given restriction enzyme between PI 437654 and BSR 101.

Enzyme	Eco RI	Hind III	Bgl II	Eco RV	Dra I	Taq I	Xba I
%	33	41	17	29	31	30	19

Total of probes included in the analysis = 100

References:

Apuya, N.R., B.L.Frazier, P.Keim, E.Roth, E.Jill, and K.G.Lark, 1988. Restriction fragment length polymorphisms as genetic markers in soybean, Glycine max (L.) Merrill. Theor. Appl. Genet. 75:889-901.

Keim, P. and R.C.Shoemaker, 1988. Construction of a random recombinant DNA library that is primarily single copy sequence. Soybean Genet. Newsl. 15:147-148.

___, T.C.Olson, and R.C.Shoemaker, 1988. A rapid protocol for isolating soybean DNA. Soybean Genet. Newsl. 15:150-152.

___, R.C.Shoemaker, and R.G.Palmer, 1989. Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet. 77:786-792.

Myers, G.O. and S.C.Anand, 1991. Inheritance of resistance and genetic relationships among soybean plant introductions to races of soybean cyst nematode. Euphytica 55:197-201.

Baltazar Baltazar M.
Pioneer Hi-Bred Int., Inc.
Plant Breeding Division
Department of Research Specialists
7250 N.W. 62nd Avenue, P.O. Box 1004
Johnston, Iowa 50131-1004

Dr. Levi Mansur
Soybean Winter Nursery
Rodriguez 1225
Los Andes, V Region, Chile

*Department of Plant Pathology
**Department of Agronomy and Plant Genetics
UNIVERSITY OF MINNESOTA
St. Paul, Minnesota 55108

1) RFLP analysis of cyst nematode resistance in soybeans

Introduction

The genetics of resistance to soybean cyst nematode (SCN), *Heterodera glycines*, has been investigated through the use of RFLP (restriction fragment length polymorphism) mapping. RFLP markers showing differences between closely related susceptible and resistant soybean lines were compared to detect markers tightly linked to genes for SCN resistance.

Strategy for Uncovering Resistance Loci

DNA from pairs of soybean lines, identical except for regions introgressed from an SCN resistant ancestor (P.I. 209332), were examined with RFLPs from throughout the soybean map. Any RFLP showing a different DNA pattern between the resistant and susceptible lines would therefore be located in one of the introgressed regions. Introgressed regions that have been maintained in several independent SCN resistant lines are likely to contain a locus for SCN resistance.

For each RFLP marker, a difference in DNA fragment pattern between a resistant line and susceptible parent indicated that the RFLP was located within an introgressed region from P.I. 209332. Those RFLPs that showed differences in all or most of the comparisons (indicating that a common region from P.I. 209332 had been retained in the breeding of independent resistant lines) were probably near SCN resistance genes.

This is illustrated in Figure 1, which shows two contrasting outcomes for the experiment. In the result shown at the top, a hypothetical RFLP marker that is always associated with the resistance phenotype is shown. In this case, RFLP differences are observed between each pair of resistant and susceptible lines. Moreover, the RFLP allele in each resistant line is identical to that observed in the source of SCN resistance (left side). By contrast, the result at the bottom shows an RFLP marker that appears to be unrelated to resistance. Here all pairs of resistant and susceptible lines have identical RFLP alleles and none are the same as the allele of the resistance source on the left.

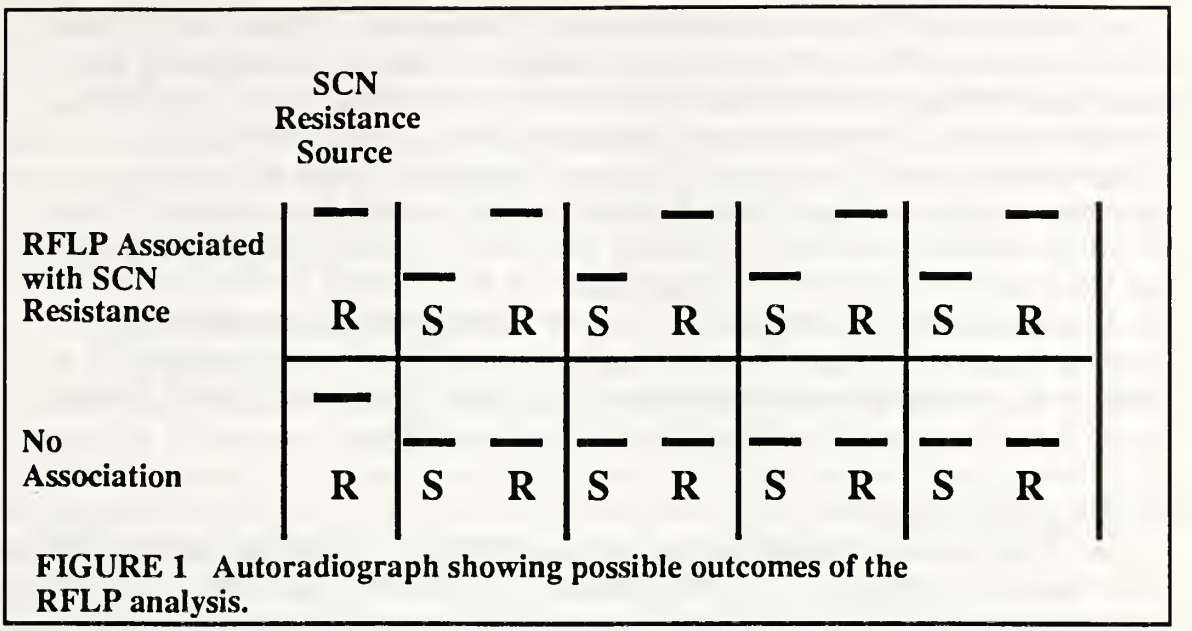


FIGURE 1 Autoradiograph showing possible outcomes of the RFLP analysis.

Materials and Methods

Four different resistant soybean lines, derived from crosses between P.I. 209332 and soybean cultivars, were utilized in this study. Abbreviated pedigrees for these lines are shown in Table I. Using these genotypes, regions of the genome derived from P.I. 209332 could be identified by comparing the RFLP pattern of each resistant line to its respective susceptible parent.

Table I

Lane No.	Line	Source	SCN Phenotype
1	209332	-----	R
2	L77-906	Williams x 209332	R
3	Evans	-----	S
4	M85-1423	Evans x L77-906	R
5	L78-169	-----	S
6	M85-1430	L78-169 x L77-906	R
7	M75-2	-----	S
8	M86-1323	M75-2 x L77-906	R
9	M75-89	-----	S
10	M86-1973	M75-89 x L77-906	R

RFLP analysis is based on the following techniques: 1) DNA extraction, 2) digestion of the DNA with restriction enzymes, 3) gel electrophoresis, 4) "Southern Blotting", 5) hybridization, and 6) autoradiography. The DNA extraction protocol from soybean leaves was based on the method of Dellaporte et al. (1983). Purified DNA was digested with six different restriction enzymes: *Hae III, Dra I, EcoR I, EcoR V, Hind III,* and *Xba I.* The resulting samples were then separated according to size by gel electrophoresis and transferred to Hybond N+ membrane by the method of Southern (1975). The filters were hybridized to DNA clones (radiolabled by the random hexamer method of Feinberg and Vogelstein, 1983) representing 60 RFLP markers from throughout the soybean RFLP map (Keim et al. 1990). These clones were the generous gift of Dr. Randy Shoemaker.

Results and Discussion

The RFLP map of the soybean genome consists of 24 linkage groups with a total length of approximately 2500 centimorgans (Keim et al. 1990). Of the 60 uniformly spaced markers used in this study, 52 were informative, which means at least one DNA length polymorphism was observed between P.I. 209332 and the cultivated lines used as parents to generate the resistant soybean lines.

Of these 52 markers, one RFLP (pK69) showed differences between the resistant line and susceptible parent four out of four times. Nine additional RFLPs showed differences three out of four times. Significantly, these RFLPs were localized to four genomic regions on four different linkage groups. These include linkage group A, linkage group B (the location of pK69), linkage group D, and linkage group S (Table II).

Table II

Outcome	Estimated Probability[a]	Number of of Clones	Linkage Groups
4/4	.000015	1	B
3/4	.0009	9[b]	A,B,D,S
2/4	.0206	11	-
1/4	.20	13	-
0/4	.77	18	-

[a] Probability of observing this result by chance.

[b] These nine RFLP markers map to four contiguous genomic regions. Therefore, they proabably indicate four independent SCN resistance loci.

In a parallel study, genomic regions retained from the SCN resistant soybean, P.I. 88788, were also examined with the RFLP markers showing putative linkage to resistance in P.I. 209332. In the case of P.I. 88788, the marker K69 was found to be associated with resistance three out of four times. This might suggest that at least one of the putative resistance genes in both lines is located in the same regions of the soybean genome. Further work is needed to confirm this hypothesis.

Detailed analysis of the resistant lines with RFLPs has also enabled us to infer the regions derived from P.I. 209332 in each line. As described above, this has been essential in identifying those regions that may be related to SCN resistance. However, this information can also be used to construct a "graphical genotype" (Young and Tanksley, 1989) for each of the derived lines. A graphical genotype for line 85-1423 is shown in Figure 2. This shows the linkage groups of soybean, with those regions derived from P.I. 209332 highlighted. Note that in addition to those genomic segments putatively associated with resistance, additional regions from P.I. 209332 have also been retained. Using RFLPs located in these regions, new and potentially superior lines that are free of P.I. 209332 germplasm in these regions can now be selected.

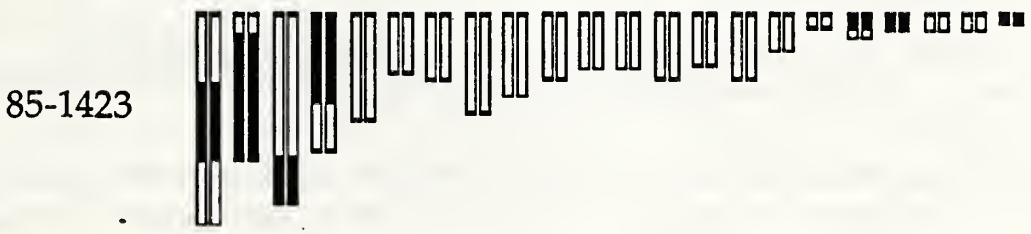


Figure 2. Graphical genotype of soybean line 85-1423. Dark regions indicate linkage blocks derived from PI 209332; light regions indicate linkage blocks from Evans.

The results described here only give an indication of the locations of SCN resistance genes. Further experiments are required to confirm these locations. In particular, F2 populations derived from crosses between resistant and susceptible lines are being examined with RFLPs and reaction to SCN. This work should proceed rapidly because only those RFLP markers associated with resistance based on these results (rather than markers throughout the genome) will need to be tested initially. Moreover, the most appropriate restriction enzyme for the analysis of each RFLP marker is already known from the experiments described here.

Acknowledgements

We would like to thank, Dr. Dariush Danesh, Dr. Christian Fatokun, Dr. Lalitha Kumar, Dr. Sharon Aarons, Dr. Desiree Menancio-Hautea, and Phil Shaus for their help in the research for this paper. This research was supported, in part, by a grant from the Minnesota Soybean Promotion and Research Council. This paper is published as a contribution in the series of the Minnesota Agricultural Experiment Station on research conducted under Project 015, supported by G.A.R. funds.

References

Dellaporte SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Pl. Mol. Biol. Rep. 1: 19-21.

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction fragments to high specific activity. Anal. Bioc. 132: 6-13.

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126: 735-742.

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503-517.

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl. Genet. 77: 95-101.

S. Boutin*
H. Ansari*
V. Concibido*
R. Denny*
J. Orf**
N. Young*

VIRGINIA STATE UNIVERSITY
P. O. Box 318
Petersburg, VA 23803

1) Response of vegetable-type soybean genotypes to acute ozone injury exposure

Introduction: Vegetable-type soybean is different from the grain-type in flavor, texture and in acceptability (Gupta and Deodhar, 1975). It is already popular as a food in the Orient and Japan. In Japan the large-seeded soybeans are used as fresh green vegetables and are cooked in the pod. They can be shelled and cooked like peas, while small-seeded soybeans are used for sprouting (Wang, 1987). Soybeans are limited in reaching their maximum yield potential in part due to environmental stress factors such as ozone (O_3). The National Crop Loss Assessment Network (NCLAN) determined that soybeans were more sensitive to O_3 than other major agricultural crops, including corn, wheat, and cotton (Heck et al., 1983). The estimated production losses from soybeans at ambient levels of O_3 ranged from 7.9 to 18.6% compared to 0.6 to 3.1% for corn (Heggestad, 1988).

Ozone produced from photochemical activity, induced by sunlight on nitrogen oxides and reactive hydrocarbons, is generally considered to be the air pollutant most injurious to crops. Damage from this pollutant can be especially severe during summer months when concentrations are sufficiently high and injure many soybean cultivars. However, the level of sensitivity to O_3 varies widely among soybean genotypes. At VSU a research program is underway to test and identify systematically vegetable-type soybeans to O_3 insensitivity.

Materials and methods: One hundred and three vegetable-type soybean genotypes, including two other cultivars, 'Essex' and 'Forrest' (O_3-insensitive and sensitive, respectively) were included as standard. Four seeds of each genotype were planted in 1.5 L plastic pot containing potting soil. Seedlings were thinned to one plant per pot after emergence. Plants were fertilized with 100 ml of Peters 20N-20P-20K (20 $g \cdot 1_{-1}$) weekly.

Twenty-one days after planting, plants were exposed to 0.6 ppm O_3 (10 times the average ambient concentrations found in Southeast United States) for two hours. Ozone exposure was initiated after plants were pre-equilibrated in the cabinet for one hour. Ozone was generated by passing O_2 through an O_3 generator and was monitored at plant canopy with Dasibi Model 1003-AH-O_3 analyzer.

One day after exposure, eight leaf discs were cut from the unifoliolate, first trifoliolate, and second trifoliolate of one plant from each genotype using 6 mm diam. borer. Electrolyte leakage was then measured using YSI Model 35 conductance meter (Yellow Spring, OH 45387). Results are presented as relative membrane leakage = conductivity of treated/conductivity of untreated. Three days after exposure plants were assessed by estimating the percentage of visible O_3 injury to the unifoliolate, first trifoliolate and second trifoliolate. Foliage was estimated on 0% to 100% scale in increments of 5%.

Results and discussion: The genotypes listed in Table 1 are ranked first based on the unifoliolate injury followed by ratings for the first trifoliolate, second trifoliolate, whole plant, and membrane leakage. Significant differences were observed for unifoliolate, first trifoliolate, second trifoliolate, and whole plant, for O_3 injury assessment, and the relative membrane leakage among the tested genotypes. The mean O_3 assessment of the most insensitive genotypes was significantly lower than the mean injury assessment of the most sensitive and two standard checks. Similarly, (Essex the O_3 -insensitive standard) had significantly lower O_3 injury assessment than the most sensitive genotypes. However, there were no significant differences within the most insensitive, sensitive, and the two standard checks. This indicates that there is genetic variability among the tested genotypes for selection and hybridization, even though the relative membrane leakage was not as sensitive as mean O_3 injury assessment. The reaction of O_3 with leaf tissue induces a disruption of cellular membranes as evidenced by increased rates of solute efflux of electrolyte leakage from the most sensitive

group (Table 1). The genotypes PI 86490, PI 86103, and PI 398479 from the most sensitive genotypes had high relative membrane leakage compared to 'Verde', 'Aoda', and PI 417310 from the most insensitive group. This study showed that acute screening can rapidly provide information on the relative sensitivity of genotypes in a short period of time. Visual assessment helped to characterize the response of the genotypes to O_3 injury. The genotypes 'Kahala', 'Green & Black', Verde, Aoda, and PI 417310 were consistently insensitive to O_3 injury. Conductivity was found as an easy, practical, and inexpensive technique to measure total electrolyte leakage. Although it is not as sensitive as visual assessment, it can still be used as additional technique to screen large amounts of genetic materials for possible O_3 insensitivity.

References:

Heggestad, H.E. 1988. Reduction in soybean seed yields by ozone air pollution. JA0C. 38:1040-1041.

Heck, W.W., R.M.Adams, W.W.Cure, A.S.Heggle, H.E.Heggestad, R.J.Kohut, L.W.Kress, J.0.Rowlings, and 0.C.Taylor. 1983. A reassessment of crop loss from ozone. Environ. Sci. Technol. 17:572A-581A.

Wang, H.L. 1987. Technical Aspects of Whole Soybean Use. p. 97-118. In: J.Dupont and E.L.0sman (ed.) Legumes in the food supply. Ames: Iowa State University press.

Gupta, A.K. and A.D.Deodhar. 1975. Trypsin inhibitor activity in soybean (Glycine max L.). Indian J. Nutri. Diet. 12:81-94.

Tadesse Mebrahtu
Wondi Mersie

Table 1. Mean visual ozone leaf injury assessment and relative membrane leakage of five most insensitive and sensitive, and two standard cultivars.

Genotype	Mean O_3 injury assessment				
	UN	FT	ST	WP	RML
	%				
Kahala	10	17	09	21	1.14
Green & Black	10	17	08	11	1.21
Verde	12	13	09	11	1.09
Aoda	13	12	12	14	1.13
PI 417310	17	23	15	24	1.02
PI 86490	81	92	91	88	2.29
PI 86103	82	84	81	80	1.67
PI 417159	84	85	81	82	1.18
PI 417359	91	93	90	91	1.22
PI 398479	91	93	93	93	1.84
Essex (cK)	48	59	58	54	1.13
Forrest(ck)	58	78	60	65	1.21
$LSD_{(0.05)}$	19	22	23	18	0.59

UN = Unifoliolate
FT = First-trifoliolate
ST = Second-trifoliolate
WP = Whole plant
RML = Relative membrane leakage
CK = CHECK

VIRGINIA STATE UNIVERSITY
Agricultural Research Station
P.O. Box 285, Petersburg, Va 23806.

1) Evaluation of immature green chinese vegetable soybean for nutrients and anti-nutritional factors

Introduction: An important part of soybean research at Virginia State University is to evaluate the nutritional quality of immature green vegetable soybean. Certainly no other vegetable can match soybeans for nutritional value. Green soybeans, for instance, have an average of 12% protein on a wet basis, compared to 7.6% for lima beans and 5.4% for peas. Vegetable soybeans are also an excellent source of unsaturated fatty acids including the Omega-3 factors. In addition, they are rich in most essential vitamins and minerals. Vegetable soybean is a popular food in the orient where the incidence of heart disease is low. Vegetable-type soybean is different from the mature grain-type soybean in flavor, and texture (Morse, 1950), and is low in trypsin inhibitors and lipoxygenase (Gupta and Deodhar, 1975; Mohamed et al., 1991; Mohamed and Rangappa, 1992). During 1990, a group of American scientists from five historically black land grant universities, who are involved in soybean research, visited several agricultural research stations in the Republic of China (Taiwan) and the People's Republic of China (PRC). They collected 25 new vegetable-type soybeans. Twenty four genotypes were successfully grown in Virginia, Alabama, Georgia, and Maryland. The objective of this study was to evaluate the nutritional value of the green immature seeds of Chinese genotypes.

Materials and methods: Twenty-three Chinese vegetable soybean genotypes were used in this study. Three replications of each entry were planted in four-row plots, arranged in randomized, complete block design, at Randolph Research Farm of Virginia State University, Petersburg, Virginia. Each four-row plot was 4 m long and 3.6 m wide, with the spacing of 0.9 m between rows, and seedling rate was 23 seeds per meter. Plants were harvested at reproductive stage 7 (R7), as described by Fehr et al. (1971), and

each entry was evaluated by harvesting the two center rows of each plot. Seed samples were analyzed for percent moisture, protein, oil, trypsin and lipoxygenase activities, according to the procedures described by AOAC (1980), Kakade et al. (1969), and Hafez et al. (1985), respectively. Data were reported on wet basis for oil and on dry meal basis for protein. Data were statistically analyzed and means were separated, by using Least Significant Difference (LSD) test at 5% level of significance.

Results and discussion: The immature seeds of Chinese vegetable soybean used in the analyses showed significant differences in percent moisture, protein and oil, and trypsin inhibitors and lipoxygenase activities. Mean percentage moisture was 68.52, 1 AC-100 genotype had the highest (75.7 %) and N2899 had the lowest (62.33%). Mean percent protein was 25.23. Genotypes G9053, 1AC-100, G10134, and AGS 129 GC84136P418 had the highest percentage of protein (28.3, 27.7, 27.7, and 27.3%, respectively) and N2862 and N1831 had the lowest (20.4 and 22.7% respectively). No significant variation in oil was found among the tested genotypes. The mean percentage oil was 6.03 on wet basis and ranged from 3.96% for N1831 to 7.41 for ZlHUA#4. No significant correlation was observed between protein and oil contents in immature seeds; however, significant and negative correlation was found between oil and moisture contents ($r = -0.430$).

Trypsin inhibitors and lipoxygenase are two of the prevalent anti-nutritional factors in soybeans. This study reveals that the mean TI activity was 423.83 TI units/mg meal and ranged from 868.7 Tl units/mg meal for AGS 292 to 261.7 TI units/mg meal for blue side. The mean TI was exceptionally higher than that for mature seeds. The accumulation of trypsin inhibitor is higher at early reproductive stages than the accumulation of storage protein. This reduces the effects of proteinases on the storage protein during maturation. The lower trypsin inhibitor activity in the mature seed is due to the accumulation of storage protein rather than the elimination of the inhibitors. The study reveals that mean lipoxygenase activity (83126 units/min/mg protein) was 2.26 times higher than that reported by Mohamed et al. (1990) and Hafez (1983)

for mature seed. That is due to low accumulation rate of storage protein at early reproductive stage. Genotypes N1 831 and N1 5989 had the highest lipoxygenase activity (107132 and 100036 units/min/mg protein, respectively), while N2899 had the lowest (45274). Lipoxygenase is responsible for the off-flavor in soybean food and beverages (Sessa, 1977) and trypsin inhibitor is responsible for poor digestibility of soyprotein. However, heating treatment of the seeds eliminates the effects of these anti-nutritional factors.

References:

AOAC. 1980. "Official Methods of Analysis", 13th ed. Association of Official Analytical Chemists. Washington, D.C.

Fehr, W.R., C.E. Caviness, D.T. Burmood, and J.S. Pennington. 1971. Development descriptions of soybean, Glycine max (L.) Merr. Crop Sci. 11:929-931.

Gupta, A.K. and A.D. Deodhar. 1975., Trypsin inhibitor activity in soybean (Glycine max (L.) Merr.). Indian J. Nutri. 12: 81-84.

Hafez, Y.S., A.I. Mohamed, G. Singh, and F.M. Hewedy. 1985. Effects of gamma irradiation on proteins and fatty acids of soybean. J. Food Sci. 50:1271-1274.

Hafez, V.S. 1983. Nutritional composition of different varieties and strains of soybean. Nutri. Report Int. 28:1197-1206.

Kakade, M.L., N. Simon, and I.E. Lenier. 1969. An evaluation of natural vs. synthetic substrate for measuring the anti-tryptic activity of soybean samples. Cereal Chem. 46:518-526.

Mohamed, A.I., T. Mebrahtu, and M. Rangappa. 1991. Nutrient composition and anti-nutritional factors in selected vegetable soybean (Glycine max (L.) Merr.). Plant Food Human Nutri. 41: 89-100.

___ and M. Rangappa. 1992. Screening soybean (grain and vegetable) genotypes for nutrients and anti-nutritional factors. Plant Food Human Nutri. 2: 87-96.

Morse, W.J. 1950. Chemical composition of soybean seeds. In Soybean Products. I.K.S. Markley (ed.), John Wiley and Sons, NY.

Sessa, D.J. 1979, Flavor consideration of soybean protein products. J. Agric. Food Chem. 27: 234-236.

Ali I. Mohamed
M. Rangappa
H. Bhardwaj
N. Pearson

Table 1. Moisture, protein (16% N), oil, trypsin inhibitor, and lipoxygenase activity in green chinese vegetable-type soybean (R7).

Genotypes	Moisture %	Protein %	Oil %	TI Units/mg Protein	Lipoxygenase Units
AGS 129	68.8	27.3	6.03	397.5	99830
AGS 269	72.0	24.6	5.83	421.9	58198
AGS 290	66.3	26.7	6.97	297.4	90212
AGS 292	66.3	25.0	6.60	868.7	86572
AGS 293	65.3	26.0	7.30	409.1	83867
AGS 314	69.0	26.0	6.51	439.1	79211
KVS 124	70.0	25.0	6.80	565.3	89832
G 9053	73.0	28.3	4.97	567.2	90380
G10134	66.0	27.7	7.00	729.7	85665
GC 84136P418	69.7	27.3	4.53	435.1	82732
1AC-100	75.7	27.7	4.93	351.7	73986
BLUE SIDE	69.0	22.4	4.27	261.7	92663
HENONG#33	67.0	24.0	7.00	270.6	74730
HEFENG#25	65.0	25.5	5.50	345.6	97015
ZIHUA#4	67.3	26.0	7.41	374.3	84189
MEINHE#3	67.0	27.2	6.30	358.3	72315
N1535-1	67.3	26.1	6.47	322.5	97485
N15989	73.3	26.3	6.53	642.3	100036
N1831	73.0	22.7	3.96	377.0	107132
N2899	62.3	25.0	5.30	280.1	45274
N2962	65.0	20.4	6.43	310.3	78109
N7788	71.3	22.8	6.28	357.4	77384
N8806	65.0	22.1	6.48	356.3	68104
CV%	3.9	8.4	6.5	14.4	14.2
LSD (0.05)	4.50	359	4.37	103.1	9672

VIRGINIA STATE UNIVERSITY
Agricultural Research Station
P.O. Box 9259, Petersburg, Va 23806.

2).Evaluation of Chinese vegetable-type soybean for nutrients and anti-nutritional factors

Introduction: Vegetable soybean is a popular food in the Orient, where the incidence of heart diseases is low. Vegetable-type soybean is different from the mature graintype soybean in flavor and texture (Morse, 1950), and is low in trypsin inhibitors and lipoxygenase (Gupta and Deodhar, 1975; Mohamed et al., 1991; Mohamed and Rangappa, 1992). Therefore, one of the goals of the current research at Virginia State University is the development of vegetable soybean cultivars with higher nutritional value. During 1990, a group of American scientists from five historically black land grant universities, who are involved in soybean research, visited several agricultural research stations in the Republic of China (Taiwan) and the People's Republic of China (PRC). They collected 25 new vegetable-type soybeans and 24 of these genotypes were successfully grown in Virginia, Alabama, Georgia, and Maryland. The objective of this study was to evaluate the nutritional value of the Chinese genotypes.

Materials and methods: Because of the limited number of seed of each genotype, seed quantities were increased at VSU in the greenhouse under controlled environmental conditions. Seeds were harvested at maturity, and a portion was used for nutritional evaluation, the other portion was planted in the field at Randolph research farm at VSU. The collected seed samples were ground and analyzed for percent protein, oil, trypsin and lipoxygenase activities as described by AOAC (1980), Kakade et al. (1969), and Hafez et al. (1985), respectively. Data were statistically analyzed and means were separated, by using Least Significant Difference (LSD) test at 5% level of significance.

Results and discussions: The Chinese vegetable-type soybean

used in the analyses showed significant differences in seed size, percent protein, oil, trypsin inhibitors, and lipoxygenase activities. Mean weight of 100 g seeds was 32 g. G101 34 genotype had the largest (51.6 g/100 seeds) and N1 535-1 had the smallest (14.8 g/100 seeds) seed size. Mean protein percent was 42.05, and AGS 314 and GC 841 36P41 8 had the highest protein (48 and 46.63 %, respectively) and AGS 129 and AGS 269 had the lowest (36.13 and 36.40% protein, respectively). The protein of the tested genotypes was lower than those genotypes reported earlier for vegetable soybean (43.32%)(Mohamed et al., 1991).

Wide variation in oil was also found. Mean oil percent was 19.11 and ranged from 13.79% for AGS 314 to 22.47 for AGS 129. The mean oil percent was higher than those values reported earlier for vegetable soybean (18.44%) by Mohamed et al. (1992). Highly significant and negative correlation (r=-0.658 **) was observed between protein and oil contents. These results are in agreement with earlier findings (Mohamed et al., 1991), and Hafez (1983). The total (protein + oil) values for genotypes AGS290, AGS 293, KVS 124, G9053,GC84136P418, BLUE SIDE, HENONG #33, HEFENG #25, ZlHUA #4, MEINHE #3, AND N1589 were higher than 62%.

Trypsin inhibitors (TI) and lipoxygenase are two of the prevalent anti-nutritional factors in soybeans. This study reveals that the mean TI activity was 25.83 TI units/mg meal and ranged from 58.03 Tl units/mg meal for GC841 36P418 to 11.32 TI units/mg meal for HENONG #33. Two genotypes (G10134 and AGS292) had exceptionally high TI activity (58.03 and 52.10 TI units/mg meal). The mean TI was similar to that reported earlier. The study also reveals that mean lipoxygenase activity (36720 units/min/mg protein) was significantly higher than that reported by Mohamed et al. (1990) and Hafez (1983). Genotype G10134 and GS 269 had the highest lipoxygenase activity (62778 and 50811 units/min/mg protein, respectively), while, N 2957-1 had the lowest (28354 units/min/mg protein). Lipoxygenase is responsible for the off-flavor in soybean food and beverages (Sessa, 1977) and trypsin inhibitor is responsible for poor digestibility of soyprotein. However, heating treatment of the seeds reduces the

effects of these anti-nutritional factors.

References:

AOAC, 1980. "Official Methods of Analysis", 13th ed. Association of Official Analytical Chemists. Washington, D.C.

Gupta, A.K. and A.D. Deodhar. 1975. Trypsin inhibitor activity in soybean (Glycine max (L.) Merr.). Indian J. Nutri. 12: 81-84.

Hafez, Y.S., A.l. Mohamed, G. Singh, and F.M. Hewedy. 1985. Effects of gamma irradiation on proteins and fatty acids of soybean. J. Food Sci. 50:1271-1274.

Hafez, Y.S. 1983, Nutritional composition of different varieties and strains of soybean. Nutri. Report Int. 28:1197-1206.

Kakade, M.L., N. Simon, and I.E. Lenier. 1969. An evaluation of natural vs. synthetic substrate for measuring the anti-tryptic activity of soybean samples. Cereal Chem. 46:518-526.

Mohamed, A.I., T. Mebrahtu, and M. Rangappa. 1991. Nutrient composition and antinutritional factors in selected vegetable soybean (Glycine max (L.) Merr.). Plant Food Human Nutri. 41: 89-100.

___ and M. Rangappa. 1992. Screening soybean (grain and vegetable) genotypes for nutrients and anti-nutritional factors. Plant Food Human Nutri. 42:87-96.

Morse, W.J. 1950. Chemical composition of soybean seeds. In: Soybean Products. I.K.S.Markley (ed.), John Wiley and Sons, NY.

Sessa, D.J. 1979. Flavor Consideration of soybean protein products. J. Agric. Food Chem. 27: 234-236.

A.I. Mohamed
M. Rangappa
H. Bhardwaj
N. Pearson

Table 1. Protein (16% N), oil, trypsin inhibitor, lipoxygenase activity, and of seed size of new chinese vegetable-type soybean genotypes.

genotypes	100 seed weight	Protein %	Oil %	Total (prot+oil) %	TI Units/ mg meal	Lipoxy-genase Units
AGS 129	28.4	36.13	22.47	58.60	15.47	48817
AGS 269	19.2	36.40	18.30	54.70	21.07	50811
AGS 290	28.0	42.87	19.60	62.47	22.05	33323
AGS 292	50.0	39.80	22.00	61.80	52.10	30493
AGS 293	47.6	40.40	21.60	62.00	21.03	32452
AGS 314	20.8	48.00	13.79	61.79	19.47	31520
KVS 124	40.8	43.17	19.23	62.40	21.24	36358
G9053	46.4	43.47	19.70	63.17	22.77	38182
G10134	51.6	40.33	21.03	61.63	58.03	62779
GC84136P418	42.4	46.63	17.30	63.93	29.53	34697
1AC-100	26.8	43.13	17.23	60.36	21.77	35633
BLUE SIDE	44.4	46.17	16.60	62.77	20.68	45447
HENONG#33	28.8	40.03	22.30	62.33	11.32	35595
HEFENG#25	29.6	43.50	19.87	63.37	28.83	35483
ZIHUA#4	30.0	40.30	22.00	62.30	24.87	37725
MEINHE#3	30.8	42.00	20.97	62.97	24.37	35246
N1535-1	14.8	40.50	18.70	59.70	24.87	33011
N1589	19.6	43.27	19.60	62.87	27.71	34949
N1831	28.0	41.50	15.60	57.10	25.09	32455
N2957-1	27.6	44.00	15.20	59.20	24.64	30579
N2899	29.2	43.27	18.27	61.84	29.53	28355
N2962	27.6	43.03	18.47	61.50	28.23	33446
N7788	20.8	38.03	20.37	58.40	21.37	31377
N8806	35.6	43.30	18.07	61.37	23.87	32548

CV %		0.94	2.14		4.84	3.35
LSD (0.05)		0.67	0.69		2.12	20750

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
Department of Plant Pathology, Physiology, and Weed Science
Blacksburg, Virginia 24061
and
RING AROUND RESEARCH
Hale Center, Texas 79041

1) Cytoplasmic diversity in Glycine soja

Introduction: Mitochondrial and chloroplast DNA restriction fragment length polymorphisms (RFLPs) provide a convenient method for determining levels of diversity in a wide variety of organisms. We have previously used a specific mitochondrial DNA to examine cytoplasmic diversity in Glycine max, and have shown that there are four major cytoplasmic groups that can be differentiated by this marker (Grabau et al., 1992). These results confirmed the previous observations that soybeans currently grown in the United States are derived from a very narrow genetic base (Delannay et al., 1983; Sisson et al., 1978). An earlier report on soybean mitochondrial DNA patterns treated Glycine soja as a uniform group (Sisson et al., 1978). We have extended our examination of soybean mitochondrial diversity to include many Glycine soja representatives, and report here the preliminary results of those studies.

Materials and methods: Mitochondrial DNA was isolated as previously described (Grabau et al., 1989). Purified mitochondrial DNA was subjected to restriction endonuclease digestion, separation by agarose gel electrophoresis, and Southern hybridization analysis (Grabau et al., 1992). The probe used in these studies was a clone containing a 2.3 kb HindIII fragment, originally obtained from the cultivar 'Williams 82'. This marker was previously shown to display the highest degree of cytoplasmic diversity among all mitochondrial DNA clones tested (Grabau et al., 1992).

Results and discussion: Table 1 shows the results of screening mitochondrial DNA from 301 Glycine soja plant introductions with the 2.3 kb HindIII probe. The three

cytoplasmic groups identified in these studies correspond to three of the four groups found in the Glycine max analysis (Grabau et al., 1992). Unusual patterns were observed in some of the samples and are designated in Table 1. These samples exhibited the "Soja-Forage-type" patterns but contained additional hybridizing fragments as well. Since we have not yet ruled out incomplete digestion or possible contamination, we have not classified these as a separate subgroup at this time. Three additional samples were found that do not fit these patterns and contained "unique" mitochondrial patterns (data not shown). The degree of diversity suggests, that, in terms of mitochondrial patterns, the species Glycine soja actually represents a set of cytoplasmic types that overlap with the types found in Glycine max. We have examined the origins of the 301 plant introductions included here and have presented a summary of the distribution of different geographical locations and cytoplasmic types in Figure 1. The cytoplasmic group identified as "Soja-Forage-type" in our previous studies was the predominant group in Glycine soja and contained members from all geographical locations (107 from Korea, 70 from China, 54 from Japan, 17 from USSR). Interestingly, the "Arksoy-type" and "Bedford-type" cytoplasms were not only less common, but showed skewed distributions with respect to their location. "Arksoy-type" cytoplasms were found only in plant introductions originating from Korea (29 samples) and Japan (8 samples), while "Bedford-type" cytoplasms were found in plant introductions from China and Korea (10 and 6 samples, respectively).

We have examined seven of the eight Glycine soja plant introductions included in a study of chloroplast diversity by (Close et al., 1989). Our results with the 2.3 kb HindIII probe were consistent with their report. PI 468.904, PI 468.905 and PI 468.906, which belonged to chloroplast group 1, also grouped together in our studies with "Bedford-type" mitochondrial patterns. Of their remaining five samples, which belonged to two different chloroplast groups, we tested four, all of which belonged to the "Soja-Forage-type" mitochondrial cytoplasmic

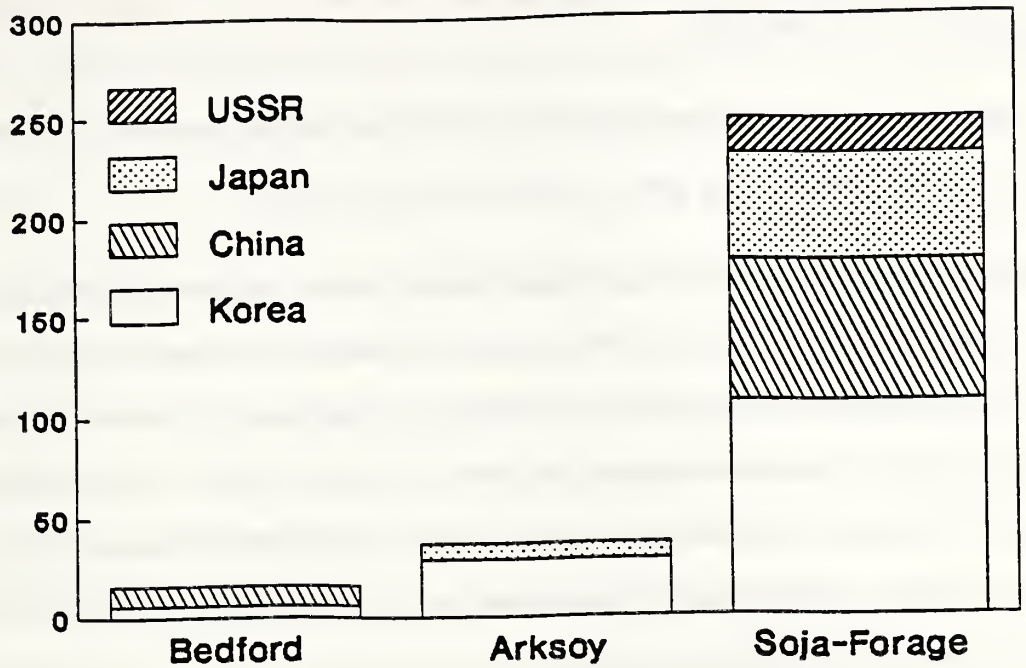
group. This result was not unexpected since we utilized only a single mitochondrial probe for our classification. It is possible that additional probes would reveal the added degree of diversity observed with the multiple chloroplast probes.

Studies currently in progress on soybean cytoplasmic diversity include a complete analysis of all 140 old domestic varieties in the soybean collection and an examination of mitochondrial diversity within Glycine soja in greater detail.

Table 1. Distribution of *Glycine soja* Plant Introductions by Cytoplasmic Type. Mitochondrial DNA was digested with HindIII and probed with a cloned 2.3 kb HindIII probe (3). * indicates samples that have been examined for chloroplast RFLP patterns (1). + indicates samples where minor bands were observed in addition to the predominant pattern.

Bedford-type	Arksoy-type	Soja-Forage-type				
407.178	339.871B	65.549	407.079	407.196	407.299	458.537A
407.180	339.871C	81.762	407.080+	407.197	407.305	458.537B
407.188	342.434	101.404A	407.081	407.198	407.308	458.539A
407.189	407.098	101.404B	407.083	407.200	407.310	458.539B
407.190	407.148	135.624	407.084	407.201	407.311	458.540A+
407.314	407.149	326.581*	407.085	407.202	407.317	458.540C+
468.904*	407.150	326.582A	407.088	407.203	407.322	458.549D+
468.905*	407.151	326.582B	407.089	407.204	423.988	464.867
468.906*	407.152	339.731	407.090	407.205	423.990A	464.868A+
468.907	407.153	339.732	407.094	407.206	423.992	464.868B
468.908	407.237	339.733	407.095	407.208	423.997	464.869B+
468.909	407.240	339.735A	407.099	407.209	423.998	464.870
468.910	407.241	339.735B	407.101	407.211	423.999B	464.871B+
468.912	407.249	339.871A	407.102	407.212	424.000	464.871C+
468.913	407.263	342.618A	407.103	407.213	424.004A*	464.889A
479.744	407.264	342.618B*	407.104	407.216	424.004B	464.889B
	407.266	342.619A	407.105	407.217	424.006B	464.889C
	407.267	342.620A +	407.126	407.218	424.007	464.890A
	407.268	342.620B	407.139	407.219	424.008A	464.890B
	407.278	342.621B	407.142	407.220	424.008B	464.891A
	424.005	349.647	407.143	407.221	424.009	464.891B+
	424.022A	366.119	407.147	407.222	424.010	464.891C+
	424.036	366.120	407.154	407.223	424.014	464.892
	424.044	366.121	407.155	407.224	424.015+	464.925B+
	424.046A	366.123	407.156	407.231	424.023+	464.925C+
	424.047	378.692	407.157	407.233	424.025A	464.927A
	424.049	378.697A	407.159	407.234	424.025B	464.927B
	424.050	378.697B	407.160	407.235	424.027A	464.927C
	424.053	378.700	407.161	407.236	424.045	468.916*
	424.054B	378.701A	407.162	407.238	424.046B	468.917
	424.056	378.701B	407.163	407.239	424.048	479.745
	424.058	391.587	407.164	407.243	424.055	479.746A
	424.061A	406.684	407.165	407.246	424.057	479.746B
	424.061B	407.018	407.166	407.247	424.059B	479.747
	424.062	407.019	407.167	407.261	424.060	479.748
	424.069	407.021	407.173	407.271	424.068	479.749
	424.070A	407.029	407.174	407.273	424.072A	479.751
		407.030	407.175	407.275	424.072B	479.753B
		407.032	407.176	407.276	424.074	479.767+
		407.033	497.177	407.277	424.075+	479.768+
		407.034	407.181	407.288+	424.076A	483.459
		407.042	407.182	407.289	424.090	483.460A
		407.046	407.183	407.290	424.092	483.460B
		407.048	407.184	407.291	424.095	483.460C
		407.071	407.186	407.292	424.099A	483.461
		407.072	407.187	407.293	440.913A	483.463
		407.074	407.191	407.295	447.003B	483.464A
		407.076	407.192	407.296	447.004	483.464B
		407.077	407.193	407.297	458.535	
		407.078	407.195	407.298	458.536+	
16 samples	37 samples	248 samples				

Figure 1. Distribution of *Glycine soja* Plant Introductions by Cytoplasmic Type and Geographic Origin.



References.

Close, P.S., J.C. Shoemaker and P. Keim. 1989. Distribution of restriction site polymorphism within the chloroplast genome of the genus Glycine, subgenus Soja. Theor. Appl. Genet. 77:768-776.

Delannay, X., D.M. Rogers and R.G. Palmer. 1983. Relative genetic contribution among ancestral lines to North American soybean cultivars. Crop Sci. 23:944-949.

Grabau, E.A., W.H. Davis and B.G. Gengenbach. 1989. Restriction fragment length polymorphism in a subclass of the 'Mandarin' soybean cytoplasm. Crop Sci. 29:1554-1559.

Grabau, E.A., W.H. Davis, N.D. Phelps and B.G. Gengenbach.1992.Classification of soybean cultivars based on mitochondrial DNA restriction fragment length polymorphisms. Crop Sci. in press.

Sisson, V.A., C.A. Brim and C.S. Levings III. 1978. Characterization of cytoplasmic diversity in soybean by restriction endonuclease analysis. Crop Sci. 18:991-996.

Elizabeth Grabau
William Davis

THE UNIVERSITY OF TENNESSEE
Department of Plant Molecular Genetics
Knoxville TN 37901-1071

1) Supernodulating soybean mutants *nts*382 and *nts*1007 map to the same RFLP marker confirming genetic complementation data.

Several supernodulating soybean mutants were isolated from the cultivar Bragg (*Glycine max* L.) background through ethyl methane sulphonate mutagenesis (Carroll *et al* 1985a,b, Gresshoff and Delves 1986). Each of these symbiotic mutants was the result of a separate mutational event. These supernodulating *nts* (nitrate tolerant symbiosis) mutants exhibit increased nodulation (up to 40 times) over their wild-type progenitor, Bragg, even in the presence of nitrate. Similar mutants were isolated by Buzzell et al. (1990), Gremaud and Harper (1989), and Kouchi and Akao (1991).

When the supernodulating mutants were crossed with the wild-type Bragg all F1 plants exhibited wild-type nodulation indicating that the *nts* gene is recessive (Delves et al 1988). The *nts* locus in the F2 population segregated as a single Mendelian gene (3 wild-type:1 supernodulating). Complementation studies were conducted on 9 of these supernodulating mutants where crosses were made in a diallel pattern (Delves *et al* 1988). No complementation was noted when the supernodulating *nts* lines were intercrossed; all F1 plants were supernodulating. This suggested that in each line the same gene was affected. One of the problems associated with interpreting these results was the inability to distinguish between the self and cross fertilized seeds which produced F1 plants. There were no morphological markers available to confirm that one half of the genotype from the male parent had been incorporated through the cross. Restriction fragment length polymorphisms (RFLPs) provide molecular markers which may be used as a genetic marker and make it possible to establish linkage between a phenotype and a particular banding pattern (Keim et al 1990, 1989).

Recent results show that the genetic locus controlling supernodulation in soybean co-segregates tightly with the cloned molecular marker pUTG-132a (Landau-Ellis et al. 1991). Our studies with RFLP linkage analysis confirm the results of Delves et al (1988) by showing that the *nts*382 and *nts*1007 alleles co-segregate with the same molecular marker.

Genetic analysis was done on the F2 populations of the supernodulating mutants (*nts*382 and *nts*1007) crossed with a distantly related soybean line *G. soja* PI468.397. Crosses were made under field conditions in the summer of 1988. In each case *G. soja*, with purple flowers, small black seeds, and vining growth habit, was used as the male parent. The female parents, mutants of the cultivar Bragg, had white flowers, buff colored seeds, and an upright growth habit. This wide cross provided indicators for confirming a successful cross. The F1 seeds were harvested and grown in the greenhouse that Fall. Plants were confirmed as true F1 hybrids by their intermediate growth habit, purple flower color and heterozygous RFLP patterns. These plants were grown to produce F2 seed.

A population of 82 F2 plants was grown from the *nts*382 X *G. soja* cross (C16); twenty of these plants were found to have the supernodulating phenotype. A population of 57 F2 plants were grown from the *nts*1007 X *G. soja* cross (A3); fifteen of these plants were found to have the supernodulating phenotype. In each of these crosses, Southern hybridization was implemented on DNA isolated from the supernodulating segregants of the F2 population. Probes were made from plasmid clones supplied by Iowa State University which detect polymorphisms between their experimental line A81-356022 (*G. max*) and PI468.916 (*G. soja*). These probes make up a large portion of a linkage map which currently covers 30 linkage groups, 293 loci, and 2400 centimorgans. We found that the *nts* locus was linked to several markers in a region of tentative linkage group E. There was approximately 10% recombination between the *nts* locus and pA-36. No

recombination was detected between pUTG-132a and the *nts* locus with our sample sizes (see Table 1).

In a total F2 population of 139 plants (combined A3 and C16) we have failed to find recombination between probe pUTG-132a and the *nts* locus. This suggests close proximity. Bragg, *nts*382, and *nts*1007 gave the same RFLP pattern when probed with pUTG-132a, indicating that the probe does not detect the mutation itself.

Table 1. Ratios of RFLP banding patterns represented in the supernodulating segregants of the F2 populations of two crosses C16 (*nts*382 X *G. soja*) and A3 (*nts*1007 X *G. soja*).

F2 Population	C16 supernodulators	A3 supernodulators	
Probe	Banding Patterns*	Banding Patterns*	Percent Recombination
pA-36	15 : 4 : 0	13 : 1 : 1	10%
pUTG-132a	20 : 0 : 0	15 : 0 : 0	0%

*Banding patterns are represented in ratios of *G. max* : heterozygous : *G. soja*.

References

Akao S, and Kouchi H 1991. A supernodulating mutant of soybean cv. Enrei induced by Ethyl-methane sulfonate (EMS). (submitted for publication)

Buzell R I, Buttery B R, and Ablett G 1990. In: Gresshoff P M, Roth L E, Stacey G, and Newton W E (eds.) Nitrogen Fixation: Achievements and Objectives. Chapman and Hall. New York. p.726.

Carroll B J, McNeil D L, and Gresshoff P M 1985a Isolation and properties of soybean (*Glycine max*) mutants that nodulate in the presence of high nitrate concentrations. Proc. Natl. Acad. Sci 82::4162-4166.

Carroll B J, McNeil D L, and Gresshoff P M 1985b A supernodulation and nitrate tolerant symbiotic (nts) soybean mutant. Plant Physiol. 78:34-40.

Delves A C, Carroll B J, and Gresshoff P M 1988. Genetic analysis and complementation studies on a number of mutant supernodulating soybean lines. J. Genet. 67: 1-8.

Gremaud M F, and Harper J E 1989. Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. Plant Physiol. 89:169-173.

Keim P, Diers B W, Olson T C, and Shoemaker R C 1990. RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126: 735-742.

Keim P, Shoemaker R C, and Palmer R G 1989. Restriction fragment length polymorphism diversity in soybean. Theor. Appl. Genet. 77: 786-792.

Landau-Ellis D, Angermüller S A, Shoemaker R C, and Gresshoff P M 1991. The genetic locus controlling supernodulation in soybean (Glycine max L.) co-segregates tightly with a cloned molecular marker. Mol. Gen. Genet. 228: 221-226.

Deborah Landau-Ellis

Peter M. Gresshoff

University of Missouri Delta Center

Portageville, MO 63873

Genetic relationships between PI 437654 and other sources of resistance to soybean cyst nematode races 3, 5 and 14.

Among the soybean plant introductions which are resistant to the soybean cyst nematodes (SCN), Peking and PI 88788 have been extensively used in cultivar development. Peking is reported to be resistant to Races 1, 3 and 5, whereas PI 88788 is resistant to Races 3 and 4 (Anand et al., 1988). In the new race classification scheme (4), the traditional Race 4 has been designated as Race 14 and will be referred as such in this note. PI 90763 is resistant to Races 1, 2, 3 and 5 (4). In the entire U.S. World Soybean collection, PI 437654 is the only line which is resistant to all known SCN races (Anand et al., 1988; Anand, 1991). This report deals with the study of genetic relationships between PI 437654 and other sources of SCN resistance which have commonly been used in cultivar development.

Materials and Methods:

Crosses were made between PI 437654 and Peking, PI 88788, PI 90763 and SCN susceptible Essex. The F_1 and F_2 population in each cross were studied to determine genetic relationships between the lines and the F_3 families were used to verify the results obtained in the F_2. Each cross was studied for reaction to Races 3, 5 and 14. The details of the screening procedures followed in this study were the same as described by Anand and Rao-Arelli (1989). The plants were scored resistant or susceptible on the basis of index of parasitism (3). Chi-square (χ^2) analyses of the data was done to test goodness of fit between observed and

expected ratios based on segregation of one or more genes.

Results and Discussion:

Race 3: All parents except Essex, used in these studies were resistant to Race 3. Populations from the crosses PI 437654 x Peking, and PI 437654 x PI 90763 did not segregate for resistance (Table 1). Thus genes conditioning resistance to Race 3 in PI 437654 are in common with Peking and PI 90763. The segregation of 13R:3S in F_2 population in the cross PI 437654 x PI 88788 indicated the presence of one dominant and one recessive gene. PI 437654 x Essex segregated 3R:61S in the F_2 population, which showed that resistance to Race 3 in PI 437654 was conditioned by one dominant and two recessive genes.

Race 5: No segregation for resistance in crosses PI 437654 x PI 90763 and PI 437654 x Peking indicated that these three parents have genes in common for resistance to Race 5 (Table 2). The cross PI 437654 x PI 88788 segregated 15R:49S in the F_2 which showed the presence of two dominant and one recessive gene conditioning resistance in PI 437654. In the cross PI 437654 x Essex, a genetic ratio of 15R:241S was observed in the F_2 indicating two dominant and two recessive gene action for resistance to Race 5.

Race 14: The cross PI 437654 x Peking gave a ratio of 1R:3S in the F_2 indicating the presence of one recessive gene, whereas the cross PI 437654 x PI 90763 showed a dominant gene in PI 437654 conditioning resistance to Race 14 (Table 3). The F_1 plants of the cross PI 437654 x PI 88788 involving both resistant parent were susceptible and gave a ratio of 1R:3S

in the F_2, suggesting one gene difference. The segregation ratio of 3R:61S in the F_2 of the cross PI 437654 x Essex showed the presence of one dominant and two recessive genes in PI 437654 conditioning resistance to Race 14.

Conclusions:

PI 437654 is very unique among the other sources of resistance such as Peking, PI 88788, and PI 90763. Its resistance to Races 3 and 5 is not different from that of Peking and PI 90763, however it has two gene differences for Race 3 and three gene differences for Race 5 compared with the genes in PI 88788. It also has one gene difference from PI 88788 for Race 14 resistance. Based on the results of all four crosses, the data indicated a four gene model with two dominant and two recessive genes conditioning resistance to Race 3. One of the dominant genes was present in PI 437654 and the other dominant gene was present in PI 88788. Similarly, for Race 5, the data fitted a four gene model with two dominant and two recessive genes. PI 437654 contained all these four genes. For race 14, both PI 437654 and PI 88788 carry a different major gene along with a minor gene for resistance.

References:

1. Anand, S.C. 1991. Registration of soybean germplasm line S88-2036 having multiple-race soybean cyst nematode resistance. Crop Sci. 31:856.

2. Anand, S.C., K.M. Gallo, I.A. Baker and E.E. Hartwig. 1988. Soybean plant introductions with resistance to races 4 or 5 of soybean cyst nematode. Crop Sci. 28:563-564.

3. Anand, S.C., and A.P. Rao-Arelli. 1989. Genetic analyses of soybean genotypes resistant to soybean cyst nematode race 5. Crop Sci. 29:1181-1184.

4. Riggs, R.D., and D.P. Schmitt. 1988. Complete characterization of the race scheme for *Heterodera glycines*. J. Nematol 20:392-395.

S. C. Anand

G. O. Myers

Table 1. Reaction of parents, F_1 and F_2 for four soybean crosses to SCN Race 3.

Parent/Cross	*Generation*	*Reaction*
PI 437654		Resistant
Peking		Resistant
PI 437654 x Peking	F_1	Resistant
PI 437654 x Peking	F_2	Resistant
PI 88788		Resistant
PI 437654 x PI 88788	F_1	Resistant
PI 437654 x PI 88788	F_2	Segregated 13R:3S
PI 90763		Resistant
PI 437654 x PI 90763	F_1	Resistant
PI 437654 x PI 90763	F_2	Resistant
Essex		Susceptible
PI 437654 x Essex	F_1	Susceptible
PI 437654 x Essex	F_2	Segregated 3R:61S

Table 2. Reaction of parents, F_1 and F_2 for four soybean crosses to SCN Race 5.

Parent/Cross	*Generation*	*Reaction*
PI 437654		Resistant
Peking		Resistant
PI 437654 x Peking	F_1	Resistant
PI 437654 x Peking	F_2	Resistant
PI 88788		Susceptible
PI 437654 x PI 88788	F_1	Susceptible
PI 437654 x PI 88788	F_2	Segregated 15R:49S
PI 90763		Resistant
PI 437654 x PI 90763	F_1	Resistant
PI 437654 x PI 90763	F_2	Resistant
Essex		Susceptible
PI 437654 x Essex	F_1	Susceptible
PI 437654 x Essex	F_2	Segregated 15R:241S

Table 3. Reaction of parents, F_1 and F_2 for four soybean crosses to SCN Race 14.

Parent/Cross	*Generation*	*Reaction*
PI 437654		Resistant
Peking		Susceptible
PI 437654 x Peking	F_1	Susceptible
PI 437654 x Peking	F_2	Segregated 1R:3S
PI 88788		Resistant
PI 437654 x PI 88788	F_1	Susceptible
PI 437654 x PI 88788	F_2	Segregated 1R:3S
PI 90763		Susceptible
PI 437654 x PI 90763	F_1	Resistant
PI 437654 x PI 90763	F_2	Segregated 3R:1S
Essex		Susceptible
PI 437654 x Essex	F_1	Susceptible
PI 437654 x Essex	F_2	Segregated 3R:61S

USDA-ARS
P.O. Box 196
Stoneville, MS 38776

Observations on possible linkage between the Rps1 locus and pod wall color

Introduction: For several years we have had an interest in gene frequency changes that might occur when segregating bulk populations of soybean that are advanced on fields naturally infested with a pathogen or the soybean cyst nematode (SCN). Hartwig et al. (1982) studied the effects of natural selection for resistance to phytophthora rot and SCN. Kilen and Keeling (1987) concluded that the frequency of genes that confer resistance to phytophthora rot could be increased if the bulk populations segregate for highly susceptible plants. This is consistent with the findings of Buzzell and Haas (1972) when they applied estimates from natural and mass selection to a theoretical bulk-hybrid population segregating for Rps and rps. In their study, they used pubescence colors to separate component varieties in the composites at time of harvest. We decided to utilize a similar approach in estimating shifts in gene frequency when composite populations were grown for several years on the slowly drained clay soils at Stoneville, MS.

Materials and methods: A cross was made between the cultivar Bedford (rps1-c, T pubescence, Tn pod wall) and D78-3173 (Rps1-c, G pubescence, Br pod wall). The F_1 plants were grown in a greenhouse and an F_2 population of about 800 plants was grown on sandy loam soil where damage from phytophthora rot has not been observed. The F_2 plants were harvested individually and pubescence color recorded. Seeds from those plants were planted in a greenhouse and the seedlings were inoculated with Race 1 of Phytophthora megasperma f. sp. glycinea (Pmg), using the hypocotyl puncture method. About 60 seeds from each of 50 lines uniformly resistant to Pmg with gray pubescence were composited. Similarly, 50 gray pubescent lines, uniformly susceptible to Pmg, were composited to form a second bulk population. From the F_2

plants with tawny pubescence, 150 lines uniformly resistant and 150 lines uniformly susceptible to Pmg were planted in single-plant progeny rows to identify lines having uniformly tawny pubescence color.

Discussion: As plants approached maturity, we observed in the plots with gray pubescence that the plot grown from uniformly Pmg-resistant lines seemed to have a high frequency of plants with brown pod wall, whereas the susceptible plot had a predominance of plants with tan pod wall. Counts of plants with brown or tan pod wall verified our observation (Table 1). We then took notes on pod wall color of the 300 plant-progeny rows with tawny pubescence. A similar trend was found (Table 1).

Our observations suggest there may be linkage between Rpsl and brown pod wall color. If this is verified, that association could be utilized to visually select a predominance of Pmg-resistant plants without screening for the disease.

Because we had discarded the lines that were segregating for Pmg and we composited lines with gray pubescence, we could not reconstruct the entire population. We have now made crosses to generate populations that will segregate for determinate and indeterminate stem, brown and tan pod wall, Rpsl-k and rpsl-k, Hm and hm and W1 and w1. This segregating material should provide the data for calculating linkage intensity between Rpsl and pod wall color. If linked, it suggests that Linkage Group 5 and Linkage Group 10 would become one linkage group.

References

Buzzell, R.I. and J.H. Haas. 1972. Natural and mass selection estimate of relative fitness for the soybean rps gene. Crop Sci. 12:75-76.

Hartwig, E.E., T.C. Kilen, L.D. Young and C.J. Edwards, Jr. 1982. Effects of natural selection in segregating soybean populations exposed to phytophthora rot or soybean cyst nematode. Crop Sci. 22:588-590.

Kilen, T.C. and B.L. Keeling. 1990. Gene frequency changes in soybean bulk populations exposed to phytophthora rot. Crop Sci. 30:575-578.

T. C. Kilen

Table 1. Number of soybean plants or lines with brown or tan pod wall color in populations resistant or susceptible to phytophthora rot.

Disease reaction	Pubescence color of F_2 plants	Pod wall color		
		Brown	Segregating	Tan
		F_3 plants		
Resistant	Gray	116	-	34
Susceptible	Gray	35	-	115
		F_3 lines		
Resistant	Tawny	61	39	51
Susceptible	Tawny	19	27	103

USDA-ARS BARC WEST
Plant Molecular Biology Laboratory
Beltsville, MD 20705
and
UNIVERSITY OF NEW HAMPSHIRE
Program in Genetics
Department of Plant Sciences
Durham, NH 03824

Test for linkage of Ap and Ti loci in Linkage Group 9 with the Dt1 and L1 loci in Linkage Group 5

Development of a classical genetic map of soybean is still at a rudimentary stage. Although there are 20 chromosome pairs, only 19 linkage groups have been clearly defined and recognized by the Soybean Genetics Committee. Most of these linkage groups consist of only two linked genes. These linkage groups have not been adequately tested for independence or linkage with each other and may, therefore, be present on the same chromosome or even overlap on the same chromosome. An example of this has recently been found in the case of the mutual linkage of Linkage Group 5 with Linkage Group 16 (Kiang, 1990). Thus, further testing of the known linkage groups is needed to establish whether they are present on the same chromosomes or on distinct chromosomes.

In this study we tested the Ap (acid phosphatase) and Ti (Kunitz trypsin inhibitor) loci, 6.6 map units apart, in Linkage Group 9 (Hildebrand et al., 1980) for linkage with the Dt1 (determinate plant growth) and L1 (pod color) loci, 39.4 map units apart, in Linkage Group 5 (Weiss, 1970). The cross of 'Toku' X L68-1562 was made in the field at Beltsville, MD. The lines Toku and L68-1562 were obtained from the USDA soybean program at Urbana, IL. L68-1562 is an isoline of 'Clark' background carrying the L1 allele for black pod. It was obtained from the USDA soybean genetics program at Urbana, Illinois. L68-1562 carries the W1, T, Ap-B, and the Ti-a alleles. Toku carries the w1, t, Ap-a, Ti-b and l1 alleles. The gene symbols given in

Table I refer to the genetics traits described by Palmer and Kilen (1987). F_2 seeds were produced in Puerto Rico during the winter. A fragment of the cotyledon of each F_2 seed was excised and used for determination of the alleles present at the Ap and Ti loci at the University of New Hampshire. After excision of the fragment, the F_2 seeds were planted in the field at Beltsville, MD and grown to maturity to determine the alleles present at the Dt1 and L1 loci. The F_2 segregation indicated a range of expression for the determinate versus indeterminate trait. Only plants that were clearly indeterminate were scored as indeterminate. Using this system of scoring, the plants heterozygous for the determinate trait were scored as determinate and, therefore, the indeterminate trait was considered recessive. The L68-1562 line was considered to carry the recessive allele for determinate growth and Toku was considered to carry the dominant allele for determinate growth. Plants were scored for black pod or non-black pod.

The method of maximum likelihood was used for all recombination estimates, except for those involving the Dt1 locus, where the product moment method was appropriate (Allard, 1956; Mather, 1951). The bisection method (Yakowitz and Szidarovsky, 1989) was used to solve the maximum likelihood equations. Chi-squares for the monogenic ratios fit a 3:1 segregation except for the Dt1 locus. Neither the Ap or Ti loci in linkage group 9 were linked to the Dt1 or L1 loci in Linkage Group 5. Therefore, no evidence of association between these two linkage groups was found. No linkage was found between the W1 locus and the T locus with the other loci studied. The linkage of Ap and Ti previously reported was confirmed. The linkage between Dt1 and L1 previously reported also was confirmed; however, our estimate of the recombination frequency was appreciably different from that of Weiss (1970). Weiss' study had the advantage of being based on 1,374 plants while the present study was based on only 359 plants. In addition, Weiss' study was conducted in coupling phase--which is more efficient in providing information, while the present study was conducted in

repulsion phase. Therefore, Weiss' study should be considered the more definitive estimate of the linkage distance.

References:

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235-278.

Hildebrand, D.F., J.H. Orf, and T. Hymowitz. 1980. Inheritance of an acid phosphatase and its linkage with the kunitz trypsin inhibitor in seed protein of soybeans. Crop Sci. 20:83-85.

Kiang, Y.T. 1990. Linkage analysis of Pgd1, Pgi1, pod color, (Li), and determinate stem (dt1) loci on soybean linkage group 5. J. Hered. 81(5):402-404.

Mather, K. 1951. The Measurement of Linkage in Heredity. 2nd. ed. Methuen & Co., London.

Palmer, R.G., and T.C. Kilen. 1987. Qualitative genetics and cytogenetics. p. 135-209. In: J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16:ASA, CSSA and SSSA, Madison, WI.

Weiss, M.G. 1970. Genetic linkage in soybeans: Linkage groups V and VI. Crop Sci. 10:469-470.

Yakowitz, S., and F. Szidarovsky. 1989. An Introduction to Numerical Computations. 2nd ed. Macmillan Publishing, New York.

T.E. Devine
Y.T. Kiang

Table 1. Summary of genetic linkage found in linkage groups 5 and 9.

Cross	Genes	Genotype* A	B	C	D	Sum	X^2 L'	P(X^2 L)§	Rec¶	SE#	Ph''	Ratio
Toku X	*Dt1, T*	224	72	32	35	363	15.7726	<.001	64.32	3.3	R	9:3:3:1
L68-1562	*Dt1, L1*	218	75	65	1	359	11.5286	<.001	16.57	5.1	R	9:3:3:1
	Dt1, W1	215	72	46	12	345	0.3095	.50-.70	46.93	4.2	R	9:3:3:1
	L1, T	195	89	60	16	360	3.5999	.05-.10	58.09	4.3	C	9:3:3:1
	L1, W1	204	65	56	17	342	0.0117	.90-.95	50.50	4.1	C	9:3:3:1
	W1, T	189	75	61	23	348	0.0460	.70-.90	50.82	4.1	C	9:3:3:1
		e+f	g+h+i	j+k	l	m	n					
	Dt1, Ap	76	145	75	17	35	15	363	0.2525	.70-.90	50.81	3.2	C	3:6:3:1:2:1
	Dt1, T1	80	147	69	15	36	16	363	0.6253	.70-.90	47.69	3.2	C	3:6:3:1:2:1
	L1, Ap	73	148	65	18	33	25	362	3.0221	.20-.30	54.66	3.2	R	3:6:3:1:2:1
	L1, T1	74	152	60	19	33	24	362	3.6703	.10-.20	54.83	3.2	R	3:6:3:1:2:1
	W1, Ap	64	134	66	24	41	19	348	0.6494	.70-.90	47.46	3.3	R	3:6:3:1:2:1
	W1, T1	64	137	63	26	40	18	348	1.4770	.30-.50	46.49	3.3	R	3:6:3:1:2:1
	T, Ap	70	130	59	23	53	31	366	2.2459	.30-.50	54.30	3.2	R	3:6:3:1:2:1
	T, T1	75	128	56	20	58	29	366	4.6739	.05-.10	55.58	3.2	R	3:6:3:1:2:1
		e	f	g	h+i	j	k	l	m	n				
	Ap, T1	118	20	19	247	0	18	1	18	119	560	698.05	<.001	7.13	0.8	C	1:2:1:2:4:2:1:2:1

* Class designations per Allard, 1956.

' Linkage chi-square tests for independence were calculated with one of three assumptions: a 9:3:3:1 ratio expected in the F2 with 1 df; a 3:6:3:1:2:1 F2 ratio with 2 df; or a 1:2:1:2:4:2:1:2:1 F2 ratio with 4 df.

§ Chi-square probability.

¶ Rec = estimate of the recombination frequency using the method of maximum likelihood.

SE = standard error of recombination estimate.

'' Phase: C = coupling; R = repulsion.

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Plant Molecular Biology Laboratory
Plant Sciences Institute
Beltsville, MD 20705

Genetic independence of the W1 and Y10 loci and the Fr2, W1, and Y12 loci

The evaluation of F_2 plants from the cross T141 X TI61 to test for genetic linkage of the W1 and Y10 loci was done in a field study at Beltsville, MD. Seedlings exhibiting foliar chlorosis (Y10 locus) in the first few weeks of growth were marked with a stake to assure proper classification should the chlorotic phenotype expressed in the seedling stage become undistinguishable with additional growth. At flowering, the plants were scored for flower color (W1 locus) and chlorosis. The evaluation of F_2 plants from the cross T233 X PI 290136 to test for the linkage of Fr2, W1, and Y12 loci was conducted in a greenhouse. After three weeks in growth trays, the seedlings were classified for hypocotyl color, controlled by the W1 locus, foliar chlorosis, controlled by the Y12 locus, and root fluorescence under UV light, controlled by the Fr2 locus.

Gene symbols given in Table 1 refer to genetic traits described by Palmer and Kilen (1987). Recombination was calculated from F_2 or F_3 data using the method of maximum likelihood as described by Allard (1956) and Mather (1951). The bisection method (Yakowitz and Szidarovsky, 1989) was used to solve the maximum likelihood equations.

Chi-square for monogenic ratios obtained for all the gene loci indicated a good fit to a 3:1 ratio. The Y10 locus segregated independently of the W1 locus in the classical soybean Linkage Group 8 (Palmer and Kilen, 1987) and Linkage Group A in the soybean RFLP map (Keim et al, 1990). The Y12 locus also segregated independently of w1 locus, as did Fr2. The Y12 locus segregated independently of the Fr2 locus.

Acknowledgments: The assistance of Shannon J. Fox, Biological Aide, Michael D. Kuykendall, Research Apprentice from Einstein High School, and Charles Marmor, a volunteer from Gonzaga College High School is gratefully acknowledged.

References

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235-278.

Keim, P., B.W. Diers, T.C. Olson, and R.C. Shoemaker. 1990. RFLP mapping in soybean: Association between marker loci and variation in quantitative traits. Genetics 126:735-742.

Mather, K. 1951. The measurement of linkage in heredity. 2nd ed. Methuen & Co., London.

Palmer, R.G., and T.C. Kilen. 1987. Qualitative genetics and cytogenetics. p. 135-209. In: J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16:ASA, CSSA, and SSSA, Madison, WI.

Yakowitz, S. and F. Szidarovsky. 1989. An Introduction to Numerical Computations. 2nd ed. Macmillan Publishing, New York.

T.E. Devine

Table 1. Soybean genetic linkage tests.

Cross	Genes	A	B	C	D	Sum	X^2 L†	P(x^2)§	Rec.¶	SE#	Ph††	Ratio
T141 x T161	*W1, Y10*	172	47	61	16	296	0.2402	.50-.70	51	4.4	C	9:3:3:1
T233 x PI290136	*Fr2, W1*	174	56	47	26	303	3.5941	.05-.10	43	5.0	C	9:3:3:1
	Y12, W1	169	62	52	20	303	0.0180	.70-.90	50	4.3	R	9:3:3:1
	Y12, Fr2	172	59	58	14	303	1.0300	.30-.50	45	4.5	R	9:3:3:1

† Linkage chi-square tests for independence were calculated with a 9:3:3:1 ratio expected in the F2 with 1 df.
§ Chi-square probability.
¶ Rec = estimate of the recombination frequency using the method of maximum likelihood.
# SE = standard error of recombination estimate.
†† Phase: C = coupling; R = repulsion.

VIRGINIA STATE UNIVERSITY
Petersburg, VA 23806
ALABAMA A&M UNIVERSITY
Normal, AL 35762
UNIVERSITY OF MARYLAND
Princess Ann, MD 21853

US DEPARTMENT OF AGRICULTURE
NORTH CAROLINA STATE UNIVERSITY
Raleigh, North Carolina 27650
NORTH CAROLINA A&T STATE UNIVERSITY
Greensboro, NC 27411

New specialty soybean germplasm from China

A USDA/OICD-sponsored agricultural, scientific, technical cooperative team visited the Republic of China (Taiwan) and the People's Republic of China (PRC) June 18-July 9, 1990 to gather information regarding soybean utilization and to increase genetic diversity of the soybean crop through new germplasm collections. A total of 25 new soybean accessions (Table 1) were collected; 12 from the Asian Vegetable Research and Development Center (AVRDC), 5 from Harbin, Heilongjiang Province, and eight from Nanjing Agricultural University, Nanjing. Those accessions were cleared for import into the US by government of China and US Quarantine System and were brought to the Virginia State University (VSU), Petersburg, VA, one of the OICD-sponsored team participants.

In accordance with the US policy on the New Germplasm Collection, part of the seed of each accession was sent in original packets to USDA Soybean Germplasm Collections, University of Illinois, Urbana, IL. in October 1990. Limited seed increase was done in the greenhouse during winter of 1991. One 10 m long observational row of each accession was grown at VSU Research Station in 1991 season. Seed traits such as seed coat and hilum color, seed size (100-seed weight in grams); plant characteristics such as hypocotyl color, plant type, flower color, pubescence color and type, pod color, pod size, pod shattering, number of seed per pod, leaf type and size, plant maturity; and insect pest and disease pathogen reactions were observed and documented. Chemical analyses for nutritional quality, anti-nutritional factors, and biochemical components of green seeds and pods at R-7 stage and mature seeds of each accession are underway and those values will be compared with

existing commercial cultivars, advanced breeding lines, and plant introductions.

The introduction of new genetic resource of soybean germplasm increases the genetic diversity of the gene pool available to breeders for developing adaptive and suitable cultivars. The germplasm diversity will improve the biological stability of the crop subjected to biotic and abiotic stress factors. Increasing the production levels, improving human nutritional quality as well as animal feeds are all possible through breeding by virtue of high nutritional values and oil composition of soybean.

Historic Perspective: China is the cradle of soybean germplasm diversity. It has made great strides, enormous contributions and continuous progress through the years in maintaining the genetic base of soybean germplasm in particular. Their soybean germplasm exploration dates back to ancient wild species to current domesticated and well adapted cultivars. China considers the germplasm exploration, maintenance, improvement through breeding, and development of desirable cultivars to be of the National Priorities. We understood that the total soybean collection in the country in 1990 was over 30,000 cultivatable genotypes and about 5,000 wild species.

Acknowledgments: We express our deep gratitude for the support of the USDA/OICD and the Agricultural Ministry of China, our sincere appreciation to the administration, faculty and staff of AVRDC and PRC Agricultural Research Stations. Our sincere thanks to Dr. Shanmugasundaram, Dr. Z.Z. Min, and Dr. J. Gai for providing us the soybean germplasm.

M. Rangappa
Virginia State University
McArthur Floyd and Val Sapra
Alabama A & M University
Jagmohan Joshi
University of Maryland
Thomas Carter USDA/ARS
North Carolina State University
M.R. Reddy
North Carolina A & T State University

TABLE-1

The following new specialty soybean germplasm accessions were collected by the 1990 USDA/OICD sponsored agricultural, scientific and technical cooperative team to china, June 18-July 10, 1990.

S. NO.	ACCESSION	SOURCES
1	AGS 129	Asia Vegetable Research and Development Center (AVRDC), Taiwan (Dr. Shanmugasundram).
2	AGS 269	" "
3	AGS 290	" "
4	AGS 292	" "
5	AGS 293	" "
6	AGS 314	" "
7	KVS 124	" "
8	G 9053	" "
9	G 10134	" "
10	GC 84136-P-4-1-8	" "
11	1AC-100	" "
12	Blue Side	" "
13	HENONG NO. 33	Helongjing Academy of Agricultral Sciences (HAAS), Harbin, China (Dr. Zhang Zeng Min).
14	HEFENG NO. 25	" "
15	ZIHUA NO.4	" "
16	MEIHE NO. 3	" "
17	ZYD-403 (79-1809)-G-SOJA	" "
	NANJIANG -SU Academy of Agricultural Sciences and Agricultural University, Nanjing, China, (Dr. j. Gai).	
18	N1535-1, Ping Nan Dou, Fujian-Summer	
19	N1589, Jin Yun Dou, Zhejiang-Summer	
20	N1831, Ping Hu Cu Huang Dou, Zhjiang-Summer	
21	N2957-1, Qui Dou 1, Hunan - Fall	
22	N2899-1, Xu Dou 2, Jiangsu-Summer	
23	N2962, Sui DAO Huang, Jiangsu, Summer	
24	N7788, Su Dou 1, Jiangsu-Summer	
25	N8806, 58-161, Jiangsu-Summer	

The above list of 25 soybean accessions are being maintance by Virginia State University, Petersburg, Va. Seed of these accessions is currently being increased and will be available for field evaluations in 1992 growing season.

CLEMSON University
Department of Agronomy and Soils
Clemson, South Carolina

New isoenzymes systems in soybeans

Introduction: Isoenzymes have been used successfully by breeders to mark agronomic traits in maize, wheat, tomato and rice (Stuber, 1989; Jaaska, 1983; Vallejos and Tanksley, 1983; Endo and Morishma, 1983). Of the 70 isoenzymes loci observed in maize and garden pea, approximately 70% were found to be polymorphic (Weeden and Wendel, 1989). In soybeans the best results for most isozyme loci were obtained from cotyledons that had been germinated for three days and prepared using a sucrose homogenizing buffer (Cardy and Beversdorf, 1983). Rennie et al. (1989) reported two electrophoretic buffer systems that provided good resolution for 16 isoenzymes. Eleven of these isoenzymes were reported to be polymorphic. Both these buffers were L-histidine-citrate but differed in their concentration of the citric acid. We have found four additional enzymes (G3PDH, TPI, ALD, and FBP) that can be resolved with these buffers. In our search for new molecular markers for gene tagging, we found improved activity and resolution for two previously reported isoenzymes (ADH and LAP) using a morpholine-citrate buffer at pH 8.3.

Isoenzyme assays: Buffer System: L-Histidine-citrate, pH 5.7: Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH) E.C.1.2.1.9 There are five forms of G3PDH in plants: three NAD-dependent G3PDH that are involved in respiration; a nuclear encoded, chloroplast-bound, NADP-dependent G3PDH, that is part of the Calvin cycle; and a cystolic-NADP dependent G3PDH that has no known function (McGowan and Gibbs, 1974). Activity was obtained for NAD-dependent enzymes; however, adequate resolution could not be obtained on the buffer systems examined. Histochemical staining specific for NADP-dependent G3PDH revealed at least two loci. The NADP-dependent loci are most probably the chloroplastic form since this enzyme is abundant and highly

active in leaves, whereas the cystolic enzyme is found in low concentrations and is relatively inactive (Kelly and Gibbs, 1973).

Zymogram patterns of two, three, four and five evenly spaced bands were found (Delorme and Skorupska, 1992). The slower locus is polymorphic. The two- and three-banded patterns are dominant. This variation at the slower locus may be due to a regulatory locus, which results in strong or weak expression of the slower locus (Delorme and Skorupska, 1992 and manuscript in preparation).

Triose-Phosphate Isomerase (TPI) E.C. 5.3.1.1: This isoenzyme is very strong in soybean cotyledons. In 24 soybean lines that were examined, no variants were observed. The zymogram pattern for TPI in soybean consists of one strong band, two moderately expressed bands and numerous weak bands (see Fig. 1). The strong TPI band appears after 5-10 min. of staining. The two moderate bands are faster and appear within 10-20 min. The weak bands appear after 30 min. of incubation, below the strong band and between the two moderately expressed. These weak loci are resolved best when an agar overlay is used.

Buffer System: L-Histidine-citrate, pH 6.5 Aldolase (ALD) E.C. 4.1.2.13: Previous papers had reported that ALD and ADH zymograms were identical (Doong and Kiang, 1987) and therefore concluded that the ALD bands were artifacts. However, an L-histidine-citrate buffer at pH 6.5 resolves two bands which do not correspond to ADH under these conditions. The slower migrating band is weak. Forty-four soybeans have been examined for ALD and only one variant (Fig. 2) in the zymogram pattern has been found.

Fructose Bisphosphate (FBP) E.C. 3.1.3.11: This isoenzyme is relatively weak in soybean cotyledons. In the 30 lines screened, one monomorphic band was observed (Fig. 3). This band migrates close to the fast ALD band and can be mistaken for that band. However, long electrophoresis times (seven hours) revealed that the FBP band was faster.

Buffer System: Morpholine-citrate pH 8.3 Alcohol Dehydrogenase (ADH) E.C. 1.1.1.1: The resolution of ADH by starch gel electrophoresis has been previously reported in soybeans (Rennie et al., 1989); however, the enzyme activity was weak. We found that the morpholine-citrate buffer system preserves greater activity and improves the mobility of the enzyme. Four strong loci were observed with this buffer system within 15 minutes (Fig. 4). Additionally, two slower, weaker loci can be observed if the gel is incubated for 1-2 hours. Expression of the slower loci was not dependable and therefore they were omitted for the designation of zymogram loci. The four strong bands included in our zymogram were consistent with the pattern reported by Rennie et al. (1989). However, based on the inheritance of the first and third bands (Fig. 4) observed in intraspecific cross and intraspecific crosses (unpublished data), we have concluded that the bands resolved on starch gels do not correlate with those reported for polyacrylamide/starch gels by Gorman and Kiang (1978).

Our laboratory has found that the manufacturer and lot of starch can affect the activity of first and third bands resulting in a loss of activity at these loci. Within cultivars, activity of the enzyme was consistent, but differences were found in enzyme activity between cultivars. The first and third bands segregate independently. Inheritance appears to be due to a single gene for each locus.

References:

Cardy, B.J., and W.D. Beversdorf. 1983. A Procedure for Starch Gel Electrophoretic Detection of Isoenzymes of Soybean. Dep. Crop. Sci. Tech. Bull. 119-8401 Univ. of Guelph, Ontario, Canada.

Delorme, R.M. and H.Skorupska. 1992. Inheritance and linkage of NADP+ glyceraldeyde-3-phosphate dehydrogenase: a genetic marker in soybeans. Abstracts of Technical Papers, 1992 Annual Meeting, Southern Branch of the American Society of Agronomy. 19:9.

Doong, J.Y.H. and Y.T.Kiang. 1987. Cultivar identification by isozyme analysis. Soybean Genet. Newsl. 14:189-226.

Endo, T. and H. Morishima. 1983. rice. In: S.D.Tanksley and T.J.Ortan [eds.], Isozymes in Plant Genetics and Breeding, part B, 129-226. Elsevier, Amsterdam.

Gorman, M.B., and Y.T.Kiang. 1978. Models for the inheritance of several variant soybean electrophoresis zymograms. J.Hered. 69:255-258.

Jaaska, V. 1983. Secale and Triticale. In: S.D.Tanksley and T.J. Ortan [eds.], Isozymes in Plant Genetics and Breeding, Part B, 79-101. Elsevier, Amsterdam.

Kelly, G.J. and M.Gibbs. 1973. Nonreversible D-glyceraldehyde 3-phosphate dehydrogenase in plant tissue. Plant Physiol. 52:111-118.

McGowan, R.E. and M.Gibbs. 1974. Comparative enzymology of glyceraldehyde-3-phosphate dehydrogenase from Pisum sativum. Plant Physiol. 54:312-319.

Rennie, B.D., M.L.Thorpe and W.D.Beversdorf. 1989. A procedural Manual for the Detection and Identification of Soybean [Glycine max (L.) Merr] Isozymes Using the Starch Gel Electrophoretic System. Tech. Bull. OAC Publication 1889, Univ. of Guelph, Ontario, Canada.

Vallejos, C.E. and S.D.Tanksley. 1983. Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor. Appl. Genet. 66:241-247.

Wendell, J.F. and N.F.Weeden. 1989. Visualization and interpretation of plant isozymes. In: D.E.Soltis and P.S. Soltis [eds.] Isozymes in Plant Biology 5-45. Dioscorides Press, Portland.

Richard M. Delorme
Halina Skorupska

Fig 1 TPI Zymogram

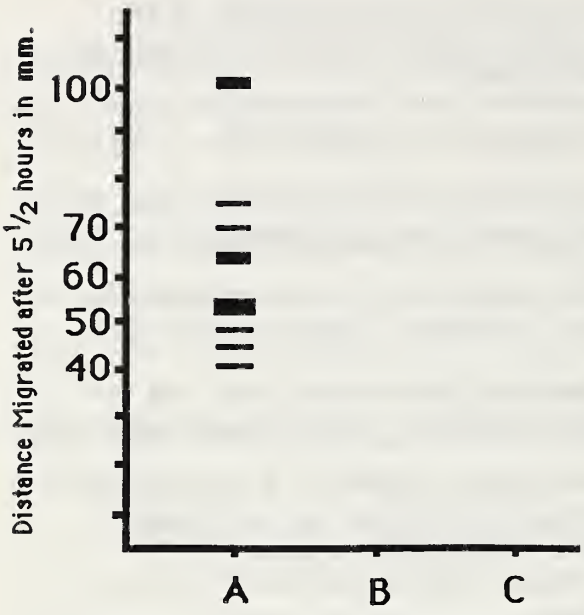


Fig 2 ALD Zymogram

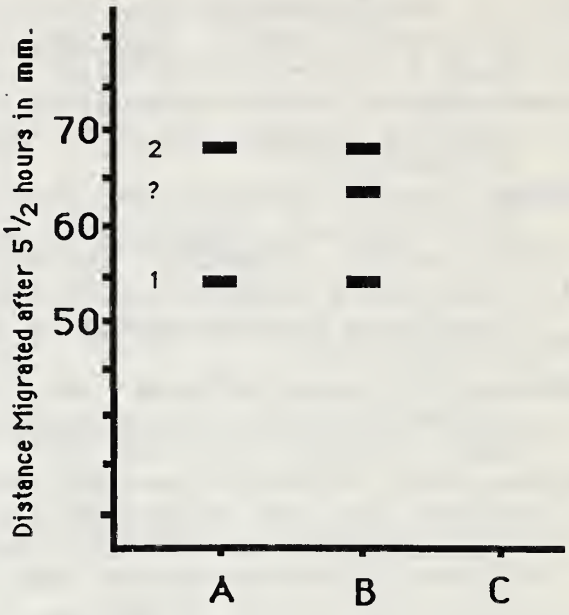


Fig 3 FBP Zymogram

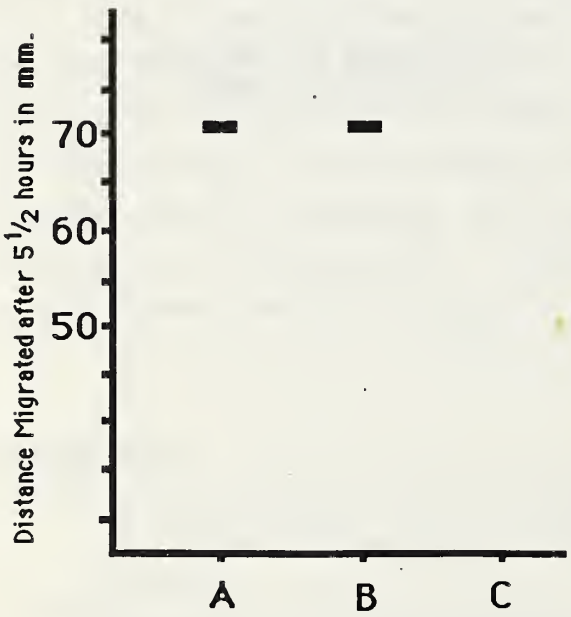
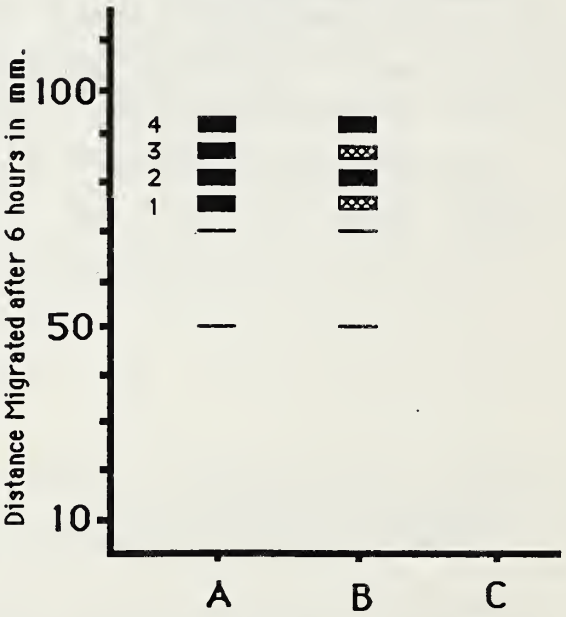


Fig 4 ADH Zymogram



Strong band — Moderate band — Weak band — Strong band inhibited by some forms of starch.

UNIVERSITY OF UTAH
Department of Biology
Salt Lake City, UT 84112

1) Molecular phylogeny as a genetic tool for soybean breeding

Phylogeny can be an important tool in plant breeding if certain conditions are met: a) the availability of an extensive germplasm; b) genetically homogeneous, nonsegregating, inbred lines; c) the availability of a sufficiently large number of polymorphic characters to differentiate individual cultivars within a species; d) a simple method for measuring these characters and determining the character state; and e) a simple method for analyzing the results. These conditions are all available for soybeans, which are inbreeding and for which an extensive, well-characterized, intra- and inter-specific germplasm already exists. Recently developed RAPD (Williams et al .,1991) markers provide an almost limitless set of characters easily analyzed by personnel with relatively little previous background in molecular biology; and computer programs, that are extremely easy to use and very informative, are now available to analyze the results. The following results, obtained in an undergraduate student laboratory illustrate this point. We present a preliminary account of the results, together with a time and cost estimate to illustrate the fact that such results are readily available to any group of scientists regardless of previous training or background.

Materials and methods We have compared four species: 11 domesticated soybeans (*Glycine max*), 9 wild soybeans (*Glycine soja*) and 5 perennials (*G. tomentella* and *G. clandestina*). PCR amplified fragments (RAPD markers (Williams et al ., 1991)) were used as characters, scored as two character states, either present or absent. Twenty-three primers (ten-mers) were used (eight were used with two different template concentrations). For each primer analysis, one reagent mixture (0.15µg ten-mer primer, $2x10^{-4}$µgm DNA template, 1µL DMSO, 1µL dNTP sol'n (5mM ea.), 2.5µL buffer(50mM $MgCl_2$, 166mM $NHSO_4$, 670mMTris HCl pH8.8), 2.5 U Taq polymerase, final volume 25 µL) was prepared and used for all 25 cultivars and all 25 reactions were treated at the same time in a thermal cycler (93 C, 45sec; 38C, 150sec; 74C, 180sec; 42 cycles). The fragments produced from the 25 reactions were separated on a single electrophoresis gel (2% agarose) and visualized by ethidium bromide staining. Fragments were analyzed from photographs of the gels. In all, the 23 primers yielded 683 characters ranging between a low of 12 to a high of 30 per primer; 590 of these characters were informative.

Table 1 lists the accessions used. Among the soybeans, five were derived from complex crosses (*), often containing some common genetic material, six were plant introductions (Bernard et al ., 1987; 1988; 1989). Among the *G. soja* , three came from a

TABLE 1

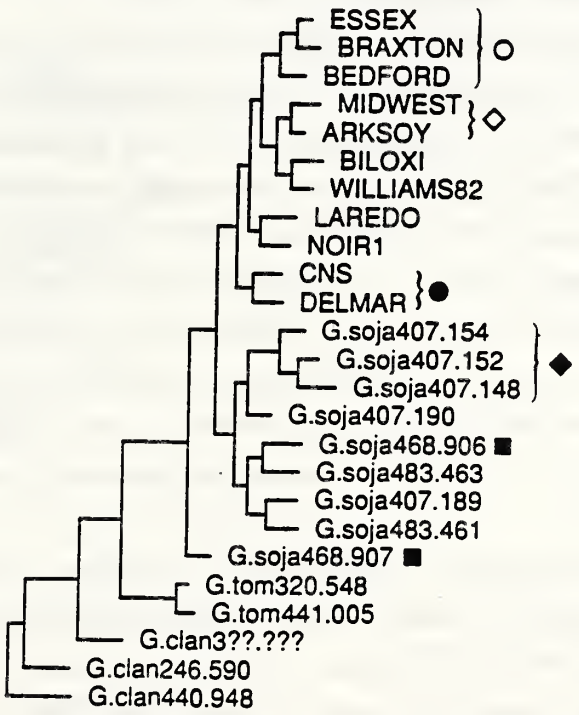
Plant Accession	Maturity Group	Origin or Pedigree
G.max		
ARKSOY (PI 37.335)	VI	Pyong Yang, Korea, 1914
BEDFORD[a]*	V	Forrest x [(Dyer x Bragg) x PI 88.788)
BILOXI (PI 23.211)	VIII	Tangxi, Zhejiang, China, 1908
BRAXTON*	VII	[Jackson x (S-100 x CNS)] x [Bragg x {(Ogden x CNS)x(Ogden x Biloxi)}]
CNS (PI 71.597)	VII	Selected from Clemson. China, 1927
DELMAR*	IV	(Patoka sib x Lincoln) x Anderson (sel. from Lincoln)
ESSEX[a]*	V	Lee x [Perry x {(Ogden x CNS) x Roanoke}
LAREDO (PI 40.658)	VI	Shensi Province, China, 1914
MIDWEST (PI 17.269)	IV	China, 1900
NOIR 1 (PI 290.136)	0	Hungary, 1963
WILLIAMS 82*	III	Williams x Kingwa
G. soja		
PI 407.148	VI	Japan (Kyushu Island), 1976
PI 407.152	VI	Japan (Kyushu Island), 1976
PI 407.154	VI	Japan (Kyushu Island),1976
PI 407.189	V	South Korea, 1976
PI 407.190	V	South Korea, 1976
PI 468.906	0	Gongzhuling Nursery, Jilin, China, 1981
PI 468.907	I	Gongzhuling Nursery, Jilin, China, 1981
PI 483.461	II	Hebei Province, China, 1979
PI 483.463	III	Shanxi Province, China, 1979
G. clandestina		c)
PI 246.590		Canberra, Australia, 1958
3??.??(PI 373.993 or PI 339.656)[b]		New South Wales, Australia, 1972 &1969 (373.993 reclassified as *G. cyrtoloba*)
PI 440.948		Canberra, Australia, 1979
G. tomentella		c)
PI 320.548		Taiwan, China, 1979
PI 441.005		Queensland, Australia, 1972

a) Bragg and Lee are descended from CNS (Bernard et al., 1988); b)The seed for this accession was mislabelled but was either PI 373.993 or PI 339.656; c) source information, personal comm. R. Nelson, University of Illinois.

small region in Kyushu, Japan. A variety of maturity groups were represented (Bernard et al ., 1989).

Results were analyzed on a Macintosh (SE30) computer using a parsimony algorithm to construct phylogenies (PAUP: Phylogenetic Analysis Using Parsimony) (Swofford and Olsen ; 1990; Swofford, 1992). This excellent program (available from D. Swofford, Illinois Natural History Survey, Champaign, IL, 61820) can be used with a personal computer,and is remarkably versatile and very user friendly. An easily executed part of this program is the ability to set reference outgroups and to add or delete taxa or characters from the analysis. An heuristic search can be accomplished within a minute or less provided the ratio of characters to taxa is sufficiently large. An exhaustive search is easily accomplished for up to 8 taxa (cultivars) within a few minutes.

Figure 1



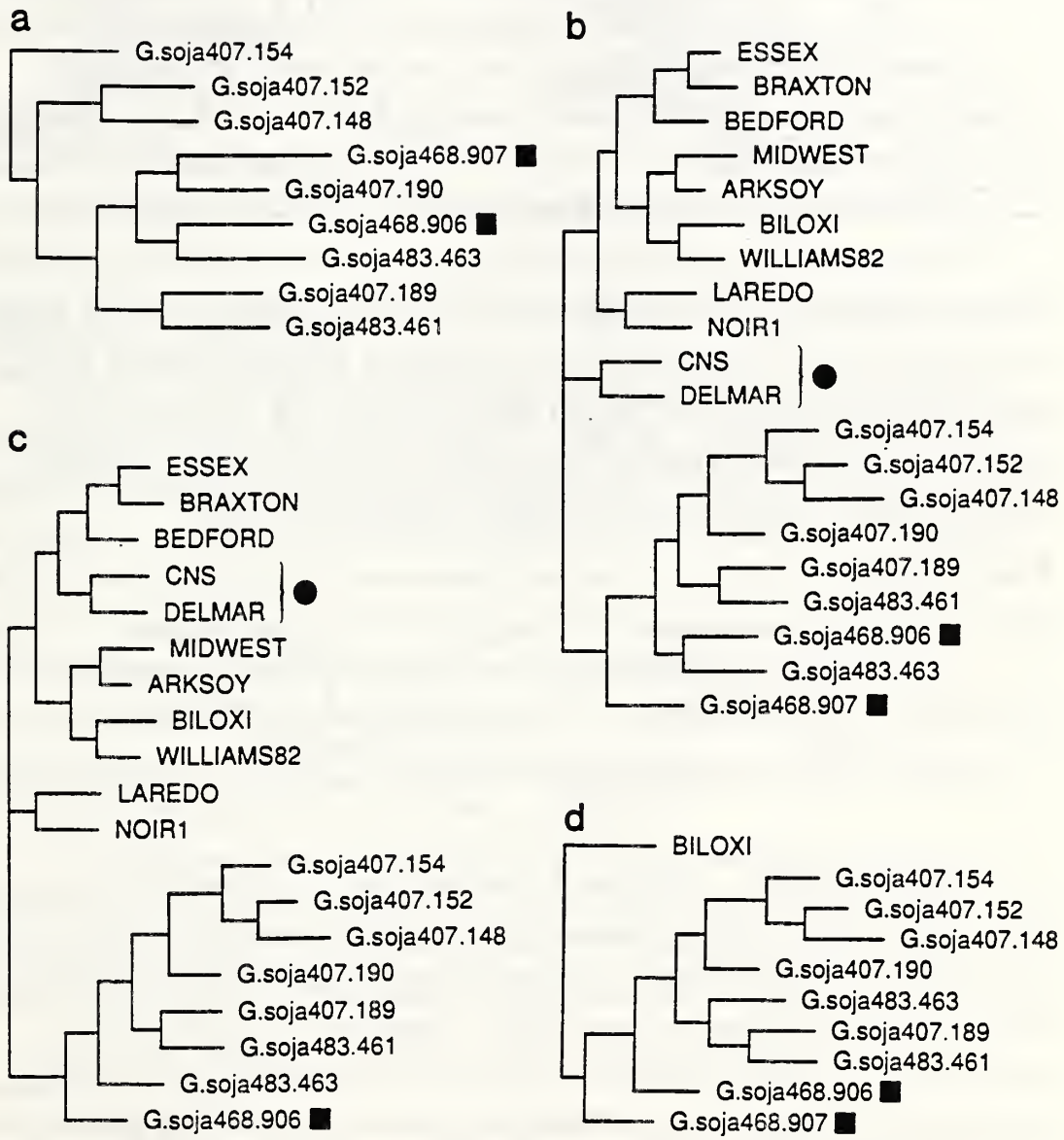
Results and Discussion Figure 1 presents a phylogram of the 25 accessions using *G. clandestina* P I 440.948 as an outgroup to polarize the phylogram. The four species *G. clandestina*, *G. tomentella*, *G. soja* and *G. max* are well separated. The three *soja* accessions — PI 407.154; PI 407.152; PI 407.148 — which originated in Japan (fig 1 ( )) are clustered together, consistent with their geographical distribution. Although the architecture of these plants differ, it seems probable that they are recently descended from a common ancestor. Again, three *G. max* cultivars, Essex, Braxton and Bedford (Fig 1( )), are clustered together consistent with the fact that they share a common genetic background (Bernard et al .,1988)). Finally, Arksoy and Midwest are paired(Fig 1 ( ))and they are morphologically extremely similar and have (among the plants used in this study) an unusually high concentration of chloroplasts in their leaves (J.Ehleringer personal comm.). Similar phylograms are obtained with the same *G. clandestina* PI 440.948 out group if only the *G. max*, *G. soja* , or the perennials are analyzed in separate phylograms (results not shown). All of these results suggest that the phylogram in figure 1 is a faithful representation of the genetic relationship of the plants involved.

In figure 1, *G. soja* PI 468.907 ( ) appears to be separated from the other *soja* accessions. When neither the perennials nor *G. max* are included in the phylogeny analysis (no out group), this cultivar is no longer separated from other *G. soja* (fig. 2a ( )). *G. soja* PI 468.907 and PI468.906 are reported to be possible descendents of interspecific hybrids between *G. max* and *G. soja* (Bernard et al.,1989). This is born out by our phylogenetic analysis (figures 2 b&c ( )). In these, PI 468.907 and PI 468.906, respectively, are closest to the *G. max* cultivars. When tested individually, 9 of the 11 *G. max* cultivars paired with one or the other of the two *max-soja* hybrid lines, preferentially selecting them from the other *soja* accessions in phylogenies containing all of the *G. soja* plus one *G. max* individual. An example of such a phylogram is shown in figure 2d.

Our analyses demonstrate that the phylograms are consistent with, and support, the known geographical or genetic information about the plants, and that parsimony analysis produces phylograms that cluster or pair plants with common genetic properties.

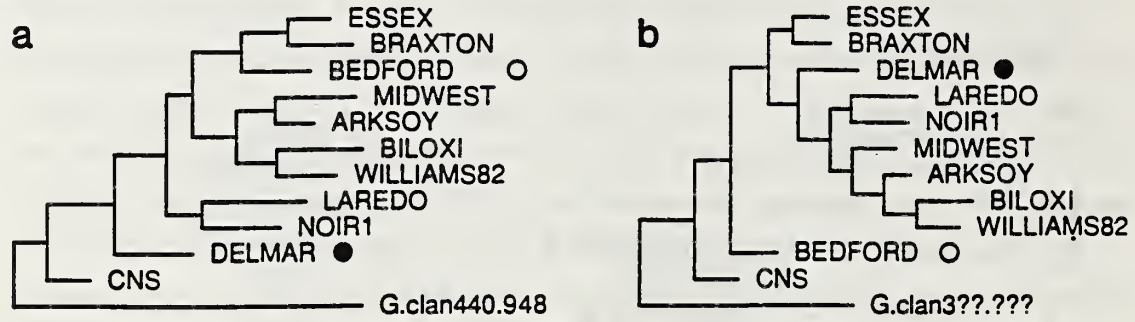
By removing plants containing a particular genetic component from the phylogenetic analysis, other genetic relationships can be revealed (compare the phylograms in figures 2a,b and c). In figure 2(b) and (c), the presence of *G. soja* PI 468.907 alters the position of two cultivars, CNS and Delmar ( ). When it is absent (fig. 2c) the phylogram indicates that CNS is related to Essex, Bedford and Braxton. The pedigrees of these plants (Bernard et al.,1988) show that they are derived from CNS. Thus, CNS has a

Figure 2


a
G.soja407.154
G.soja407.152
G.soja407.148
G.soja468.907
G.soja407.190
G.soja468.906
G.soja483.463
G.soja407.189
G.soja483.461
b
ESSEX
BRAXTON
BEDFORD
MIDWEST
ARKSOY
BILOXI
WILLIAMS82
LAREDO
NOIR1
CNS
DELMAR
G.soja407.154
G.soja407.152
G.soja407.148
G.soja407.190
G.soja407.189
G.soja483.461
G.soja468.906
G.soja483.463
G.soja468.907
c
ESSEX
BRAXTON
BEDFORD
CNS
DELMAR
MIDWEST
ARKSOY
BILOXI
WILLIAMS82
LAREDO
NOIR1
G.soja407.154
G.soja407.152
G.soja407.148
G.soja407.190
G.soja407.189
G.soja483.461
G.soja483.463
G.soja468.906
d
BILOXI
G.soja407.154
G.soja407.152
G.soja407.148
G.soja407.190
G.soja483.463
G.soja407.189
G.soja483.461
G.soja468.906
G.soja468.907

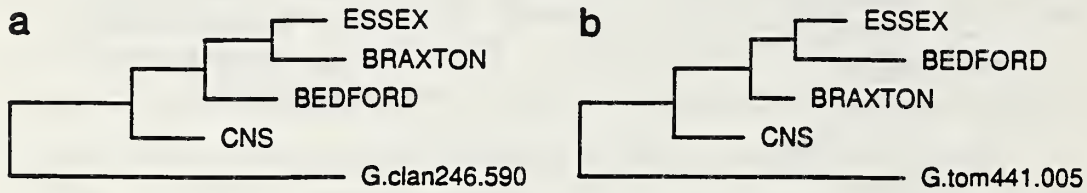
strong genetic relationship to Delmar on the one hand, and to its own descendents on the other. This dual relationship also can be demonstrated by including different *G.clandestina* accessions in the phylogenetic analysis (fig. 3).

Figure 3



With one, CNS is distant from Bedford ( ), but close to Delmar ( ). With the other, the opposite is true. Presumably the two *G. clandestina* accessions differ, in that one shares genomic characters with CNS and Delmar the other with CNS and Bedford. A prediction of these results is that CNS must share a large portion of its genome with Lincoln, the most important ancestor of Delmar (Bernard et al 1988).

Figure 4



The three cultivars — Bedford, Braxton and Essex — all have CNS as an ancestor (Bernard et al., 1988). We have already noted that CNS shares some character states with the perennials. We can use these to discriminate between the cultivars derived from CNS. CNS shares a group of character states with *G. clandestina* PI 246.590. With *G. clandestina* PI 246.590 as an outgroup, Bedford is phylogenetically close to CNS suggesting that this descendent inherited these characters from CNS (fig. 4a). In contrast, Braxton may have inherited character states identical to those in *G. tomentella* PI 320.548 (fig. 4b). Because relationships are determined by common character states, pairing of plants always will be determined by the other genomes present in the phylogeny and by the primers used to define the set of characters. When primers are chosen at random (as here) relationships nevertheless can be revealed by adding or deleting taxa as in figures 3 & 4.

A surprising result of our analysis was the unexpectedly close relationship between Biloxi and Williams 82. Most of our efforts to separate these two cultivars failed. However, they could be distinguished on the basis of their relationship to particular *G. soja* accessions. Biloxi is closely related with *G. soja* PI 468.907; PI 468.906; and PI 483.463, whereas Williams 82 is not (data not shown). However Biloxi and Williams 82 have sufficient genomic relatedness, that this difference can only be shown if they are added to the *G. soja* separately. (Compare fig. 2b with fig. 2d.)

Implications We believe that analyses of this type can be of invaluable help to breeders interested in the genetic backgrounds of plants from which they hope to derive characters of agronomic interest. If it can be shown that a particular quantitative trait is commonly associated with a well characterized phylogenetic association (such as the association of Braxton with *G. tomentella* PI 441.005 (fig 4) this relationship could become a valuable diagnostic tool for screening germplasm or following a particular character in a breeding program.

Our results were obtained by untrained undergraduate students who required approximately 80 man hours to prepare the DNA from the 25 cultivars and accessions (a single individual could probably do it in 40 hrs); and 400 man hours to obtain the RAPD results (including PCR cycling and analyzing the fragments by agarose electrophoresis). This labor estimate is high and could probably be cut in half without the restrictions imposed by a classroom format. The major cost of materials was for the PCR reagents, ca $1500. The equipment required for thermal cycling (Hybaid thermal cycler), gel electrophoresis and computer analysis (Macintosh SE) was about $6,000. We believe that any group can afford to use this genetic approach as a supplement to other analyses of cultivars and accessions to be used for breeding purposes.

For routine use, it would be advisable to include standard plants in order to define standard phylogenetic relationships in each analysis. Because very small quantities of plant DNA are required for PCR (our students prepared 200 micrograms from each plant, enough for 10^6 reactions), DNA samples can be shared between laboratories, facilitating the use of different plants (other perennial species, *G. soja* and certain domesticated soybeans) as standards.

Acknowledgements This research was carried out in Biology 360, an undergraduate laboratory on Molecular Evolution, part of an undergraduate initiative supported by a grant from the Howard Hughes Medical Institute to the department of Biology. Seeds for the various plant accessions were a gift from Ring-Around Seed Co., and were provided by Drs. Bill Davis and Norris Phelps. The accessions we used were suggested by Dr. Elizabeth Grabau and comprised four different cytoplasmic genetic groups.

References

Bernard, R. L., G.A.Juvik, and R.L. Nelson. 1987. USDA Soybean Germplasm Collection Inventory 1, International Agricultural Publications, INTSOY Series #30.

Bernard, R. L., G.A. Juvik and R.L. Nelson. 1989. USDA Soybean Germplasm Collection Inventory 2, International Agricultural Publications, INTSOY Series #31.

Bernard, R.L., G.A. Juvik, E.E. Hartwig, and C.J. Edwards, Jr., 1988. Origins and Pedigrees of Public Soybean Varieties in the United States and Canada, USDA T echnical Bulletin #1746.

Williams,, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey.1991. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Research 18 6531-6535.

Swofford, D.M. Manual for Paup version 3.0.

Swofford, D.L. and Olsen, G.J. Phylogeny reconstruction, pp 411-501 in Molecular Systematics, D.M. Hillis and C. Moritz (eds), Sinauer, Mass.

K. G. Lark*
J. Evans**
F. Basha***
R. Bogden***
R. Copeland***
R. Ellison***
D. Horne***
K. Lee***
K. McDonald***
J. Pierson***
W. Schuster***
P. Wilhelm***
Y. Yu***

Faculty*, graduate teaching assistant** students***

Department of Agronomy
UNIVERSITY OF ILLINOIS
Urbana, IL 61801

1) Cytological standards for the wild perennial Glycine

Table 1 contains a list of the wild perennial Glycine species, the somatic chromosome number, IL number and PI number of accessions used as cytological standards. The important points are as follows:

1. For genomic studies a standard set of cytological standards must be utilized.
2. If a standard for a species is crossed to another accession of the same species and perfect chromosome pairing is observed, then the second accession can be used as a standard.
3. Currently we are working on the genomic relationships of four species. Thus no standards have been assigned to these species.

References

Constanza,S.H. and T.Hymowitz. 1987. Adventitious roots in Glycine subg. Glycine (Leguminosae): morphological and taxonomic indicators of the B genome. PI. Syst. Eval. 158:37-46.

Doyle,J.J., J.L.Doyle, and A.H.D.Brown 1990a. A chloroplast-DNA phylogeny of the wild perennial relatives of soybean (Glycine subgenus Glycine): Congruence with morphological and crossing groups. Evolution 44:371-389.

___, ___, and ___. 1990b. Chloroplast DNA phylogenetic affinities of newly described species in Glycine (Leguminosae: Phaseoleae). Syst. Bot. 15:466-471.

Singh,R.J. and T.Hymowitz. 1985a. The genomic relationships among six wild perennial species of the genus Glycine subgenus Glycine Willd. Theor. Appl. Genet. 71:221-230.

___ and ___. 1985b. Intra- and interspecific hybridization in the genus Glycine, subgenus Glycine Willd: Chromosome pairing and genome relationships. Z. Pflanzenzuchtg. 95:289-310.

___, K.P.Kollipara and T.Hymowitz. 1988. Further data on the genomic relationships among wild perennial species (2n=40) of the genus Glycine Willd. Genome 30:166-176.

___, ___, and ___. n.d. Genomic relationships among diploid wild perennial species of the genus Glycine Willd. subgenus Glycine revealed by cytogenetics and seed protein electrophoresis. Theor. Appl. Genet. (in review).

___, ___, F.Ahmad and T.Hymowitz. n.d. Putative diploid ancestors of 80-chromosome Glycine tabacina (Labill.) Benth. Genome (in press).

R. I. Singh and T. Hymowitz

Table 1. *Glycine* species, somatic chromosome number, IL number, and PI number of accessions used as cytological standards, 1991.

Species	2*n*	IL*	PI
1. *G. albicans* Tind. and Craven	40	-----	---------
2. *G. arenaria* Tind.	40	-----	---------
3. *G. argyrea* Tind.	40	0768	505.151
4. *G. canescens* F. J. Herm.	40	0401	440.928
5. *G. clandestina* Wendl.	40	0425	440.948
6. *G. curvata* Tind.	40	0791	505.166
7. *G. cyrtoloba* Tind.	40	0481	440.963
8. *G. falcata* Benth.	40	0674	505.179
9. *G. hirticaulis* Tind. and Craven	80	-----	---------
10. *G. lactovirens* Tind. and Craven	40	-----	---------
11. *G. latifolia* (Benth.) Newell and Hymowitz	40	0373	378.709
12. *G. latrobeana* (Meissn.) Benth.	40	0659	483.196
13. *G. microphylla* (Benth.) Tind.	40	0449	440.956
14. *G. tabacina* (Labill.) Benth.	40	0370	373.990
no adventitious roots	80	0506	440.996
with adventitious roots		0640	483.204
15. *G. tomentella* Hayata	38	0398	440.998
	40	0614	446.993
	78	0363	339.657
	80	0485	441.005

* Temporary number assigned to an accession at the University of Illinios until a permanent PI number is assigned by the USDA to the accession.

2) Biosystems of the Genus Glycine 1991.

Table 1 contains the three-letter code, somatic chromosome number, genome symbol, and distribution of each species in the genus Glycine.

The main points are as follows:

1. Glycine curvata has been assigned to the genome symbol C_1C_1.
2. G. latrobeana has been assigned to genome symbol A_3A_3.
3. G. tabacina (2n+80) is a complex containing accessions with A and B genomes (no adventitious roots; genomic allopolyploid) or two B genomes (with adventitious roots; segmental allopolyploid).
4. G. tomentella (2n=40) appears to consist of at least three genomic groups. Currently, we are studying the diversity in this cytotype.
5. G. tomentella (2n=78, 80) are complexes. Currently we are attempting to sort out the genomic makeup of each cytotype.

References

Costanza, S.H. and T. Hymowitz. 1987. Adventitious roots in Glycine subg. Glycine (leguminosae): morphological and taxonomic indicators of the B genome. Pl. Syst. Evol. 158:37-46.

Doyle, J.J., J.L. Doyle, and A.H.D. Brown 1990a. A chloroplast-DNA phylogeny of the wild perennial relatives of soybean (Glycine subgenus Glycine) Congruence with morphological and crossing groups. Evolution 44:371-389.

___,___ and ___. 1990b. Chloroplast DNA phylogenetic affinities of newly described species in Glycine Willd. Theor. Appl. Genet. 71:221-230.

___ and ___. 1985b. Intra- and interspecific hybridization in the genus Glycine, subgenus Glycine Willd: Chromosome pairing and genome relationships. Z. Pflanzenzuchtg. 95:289-319.

___, K.P. Kollipara and T. Hymowitz. 1988. Further data on the genomic relationships among wild perennial species (2n=40) of the genus Glycine Willd. Genome 30:166-176.

___, ___ and ___. n.d. Genomic relationships among diploid wild perennial species of the genus Glycine Willd. subgenus Glycine revealed by cytogenetics and seed protein electrophoresis. Theor. Appl. Genet. (in review).

___, ___, F. Ahmad and T. Hymowitz. n.d. Putative diploid ancestors of 80-chromosome Glycine tabacina (Labill.) Benth. Genome (in press).

T. Hymowitz
R.J. Singh

Table 1. Species in the genus *Glycine* Willd., three letter code, somatic chromosome number, genome symbol, and distribution, 1991.

Species	Code	2*n*	Genome	Distribution
Subgenus *Glycine* (*x*=10)				
1. *G. albicans* Tind. and Craven	ALB	40	--	Australia
2. *G. arenaria* Tind.	ARE	40	--	Australia
3. *G. argyrea* Tind.	ARG	40	A_2A_2	Australia
4. *G. canescens* F. J. Herm.	CAN	40	AA	Australia
5. *G. clandestina* Wendl.	CLA	40	A_1A_1	Australia
6. *G. curvata* Tind.	CUR	40	C_1C_1	Australia
7. *G. cyrtoloba* Tind.	CYR	40	CC	Australia
8. *G. falcata* Benth.	FAL	40	FF	Australia
9. *G. hirticaulis* Tind. and Craven	HIR	80	--	Australia
10. *G. lactovirens* Tind. and Craven	LAC	40	--	Australia
11. *G. latifolia* (Benth.) Newell and Hymowitz	LAT	40	B_1B_1	Australia
12. *G. latrobeana* (Meissn.) Benth.	LTR	40	A_3A_3	Australia
13. *G. microphylla* (Benth.) Tind.	MIC	40	BB	Australia
14. *G. tabacina* (Labill.) Benth.	TAB	40	B_2B_2	Australia
		80	Complex[1]	Australia, West Central and South Pacific Islands
15. *G. tomentella* Hayata	TOM	38	EE	Australia
		40	DD[2]	Australia, Papua New Guinea
		78	Complex[3]	Australia, Papua New Guinea
		80	Complex[4]	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
Subgenus *Soja* (Moench) F. J. Herm. (*x*=10)				
16. *G. soja* Sieb. and Zucc.	SOJ	40	GG	P.R.C., U.S.S.R., Taiwan, Japan, Korea (Wild Soybean)
17. *G. max* (L.) Merr.	MAX	40	GG	Cultigen (Soybean)

[1] Allopolyploids (A and B genome species) and segmental allopolyploids (B genome species).
[2] At least three distinct genomic groups.
[3] Allopolyploids (D and E, A and E, or any other unknown combination).
[4] Allopolyploids (some accessions contain A and D genome species).

3) Management of the USDA wild perennial Glycine collection, 1991

Under a specific cooperative agreement between the University of Illinois and the USDA, the wild perennial Glycine collection is being maintained at the University of Illinois. Table 1 contains the names of the species, somatic chromosome number and number of accessions. The major points are as follows:

1. Four species, G. albicans, G.hirticaulis. G.lactovirens, G.latrobeana, as well as accessions of other species are recalcitrant regarding seed multiplication. With the assistance of Dr. Henry L. Shands and Dr. Antonio Sotomayor of the USDA, we are attempting to multiply certain Glycine accessions at the USDA Tropical Agricultural Research Station, Mayaguez, Puerto Rico. Thus far, 15 accessions successfully were multiplied.
2. During the year, 9 seed requests were received. A total of 258 packets of seed were shipped. Most packets had 5 to 10 seeds per accession. Domestically, seed were shipped to Alabama, California, Colorado, Indiana and Puerto Rico. Internationally, seed were sent to Australia, Peoples Republic of China and Indonesia.
3. Voucher specimens of all accessions grown out have been placed in the Crop Evolution Herbarium (CEL).
4. Approximately 84 wild perennial Glycine accessions, each containing 50 seed per packet, were sent to the National Seed Storage Laboratory, Fort Collins, Colorado. Another emergency set containing 10 seed packets was sent to Dr. R. L. Nelson, Curator, USDA Soybean Germplasm Collection, Urbana, IL. Currently, the wild perennial Glycine accessions are maintained in envelopes in a milk cooler, set at 4°C, located in C-117 Turner Hall. Thus far, about 475 wild perennial Glycine accessions have been sent to the National Seed Storage Laboratory. Many of the accessions being multiplied need to have permanent PI numbers assigned to them.
5. The inventory list is updated on a day-to-day basis utilizing a IBM PS/2 Model 70 computer.
6. A total of six new accessions were received from

CSIRO/Canberra, Australia. All of the new accessions were collected in Australia.

7. As usual, it is a constant battle to prevent pests from severely damaging the perennial Glycine plants grown either in the Turner Hall greenhouse complex or just outside the greenhouse. The following control measures were utilized:

a. Mealy bugs: Insecticidal soap, Orthene, Diazinon, Knox-Off, 100% ethanol and cotton swabs, Mavrik and Enstar.
b. Spider mites: Avid.
c. White flies: Orthene, Exclude, Yellow sticky strips, Tame.
d. Mildews: Benomyl.
e. Fungus: Banrot.

Other chemicals used included malathion and Sevin (outside the greenhouse for bean beetles). Jean Burridge passed the State of Illinois Chemical Applicators Exam and dons safety equipment as per state regulations.

In October, the greenhouse was thoroughly cleaned and fumigated with Sumithren. All clay pots and bamboo stakes are steamed. The sand bench used for germinating seed was thoroughly drenched with Banrot.

8. To request seed please write to T. Hymowitz, Department of Agronomy, University of Illinois, 1102 South Goodwin Ave., Urbana, IL 61801, USA.

T. Hymowitz
J.A. Burridge

Table 1. Wild perennial *Glycine* species, somatic chromosome number, and number of accessions, 1991.

Species	2*n*	Number of Accessions
1. *G. albicans* Tind. and Craven	40	1*
2. *G. arenaria* Tind.	40	3
3. *G. argyrea* Tind.	40	3
4. *G. canescens* F. J. Herm.	40	56
5. *G. clandestina* Wendl.	40	129
6. *G. curvata* Tind.	40	5
7. *G. cyrtoloba* Tind.	40	28
8. *G. falcata* Benth.	40	16
9. *G. hirticaulis* Tind. and Craven	80	1*
10. *G. lactovirens* Tind. and Craven	40	1*
11. *G. latifolia* (Benth.) Newell and Hymowitz	40	44
12. *G. latrobeana* (Meissn.) Benth.	40	12*
13. *G. microphylla* (Benth.) Tind.	40	24
14. *G. tabacina* (Labill.) Benth.	40	19
	80	86
	?	143
15. *G. tomentella* Hayata	38	4
	40	15
	78	54
	80	54
	?	145

* Recalcitrant species with regard to seed multiplication.

INDEX OF AUTHORS

RECENT SOYBEAN GENETICS AND BREEDING PUBLICATIONS

Ablett,-G.R.; Beversdorf,-W.D. 1991. RCAT Persian soybean. Can. J. Plant Sci. Rev. Can. Phytotech. 71:175-176.

Adcock,-T.E.; Banks,-P.A. 1991. Effects of preemergence herbicides on the competitiveness of selected weeds. Weed Sci. 39:54-56.

Adlof,-R. 1991. Fractionation of egg and soybean phosphatidylcholines by silver resin chromatography. J. Chromatogr. 538:469-473.

Akada,-S.; Kung,-S.D.; Dube,-S.K. 1991. The nucleotide sequence of gene 1 of the soybean chalcone synthase multigene family. Plant Mol. Biol. Int. J. Fund. Res. Genet, Eng. 16:751-752.

Akey,-W.C.; Jurik,-T.W.; Dekker,-J. 1991. A replacement series evaluation of competition between velvetleaf (Abutilon theophrasti) and soybean (Glycine max). Weed Res. 31:63-72.

Allen,-L.H. Jr.; Bisbal,-E.C.; Boote,-K.J.; Jones, -P.H. 1991. Soybean dry matter allocation under subambient and superambient levels of carbon dioxide. Agron. J. 83:875-883.

Alston,-D.G.; Bradley,-J.R.-Jr.; Schmitt,-D.P.; Coble,-H.D. 1991. Relationship of Heliothis zea predators, parasitoids and entomopathogens to canopy development in soybean as affected by Heterodera glycines and weeds. Entomol. Exp. Appl. 58:279-288.

Alva,-A.K.; Edwards,-D.G.; Asher,-C.J. 1991. Effects of acid soil infertility factors on mineral composition of soybean and cowpea tops. J. Plant Nutr. 14:187-203.

Anand,-S.C.; Myers,-G.O. 1991. Development of cyst nematode-resistant soybean population S89-3151. Crop Sci. 31:1371.

Anderson,-J.M. 1991. Jasmonic acid-dependent increase in vegetative storage protein in soybean tissue cultures. J. Plant Growth Regul. 10:5-10.

Anthamatten,-D.; Hennecke,-H. 1991. The regulatory status of the fixL- and fixJ-like genes in Bradyrhizobium japonicum may be different from that in Rhizobium meliloti. MGG Mol. Gen. Genet. 225:38-48.

Ashenafi,-M.; Busse,-M. 1991. The microflora of soak water during tempeh production from various beans. J. Appl. Bacteriol. 70:334-338.

Ashenafi,-M.; Busse,-M. 1991. Growth of Bacillus cereus in fermenting tempeh made from various beans and its inhibition by Lactobacillus plantarum. J. Appl. Bacteriol. 70:329-333.

Balatti,-P.A.; Krishnan,-H.B.; Pueppke,-S.G. 1991. Calcium regulates growth of Rhizobium fredii and its ability to nodulate soybean cPeking. Can. J. Microbiol. 37:542-548.

Barthou,-H.; Buis,-R. 1991. Kinetics of apical phyllogenesis of the main stem of soybean (Glycine max (L.) Merr.): effects of stand density. Environ. Exp. Bot. 31:59-66.

Bauer,-T.A.; Mortensen,-D.A.; Wicks,-G.A.; Hayden,-T.A.; Martin,-A.R. 1991. Environmental variability associated with economic thresholds for soybeans. Weed Sci. 39:564-569.

Benveniste,-I.; Lesot,-A.; Hasenfratz,-M.P.; Kochs,-G.; Durst,-F. 1991. Multiple forms of NADPH-cytochrome P450 reductase in higher plants. Biochem. Biophys. Res. Commun. 177:105-112.

Bergmann,-J.E.; Preddie,-E.; Cortes,-L.; Brousseau,-R. 1991. A protein drp90 encoded on the leftwards strand of soybean nodule urate oxidase cDNA binds to a regulatory sequence in leghemoglobin C3 gene. Nucleic Acids Res. 19:1338.

Bernard,-R.L.; Hymowitz,-T.; Cremeens,-C.R. 1991. Registration of 'Kunitz' soybean. Crop Sci. 31:232-233.

Bernard,-R.L.; Noel,-G.R. 1991. Registration of 'Linford' soybean. Crop Sci. 31:232.

Bernard,-R.L. 1991. Registration of 'Il2' soybean. Crop Sci. 31:234.

Bernard,-R.L. 1991. Registration of 'Il1' soybean. Crop Sci. 31:233-234.

Bhadula,-S.K.; Shargool,-P.D. 1991. A plastidial localization and origin of L-glutamate dehydrogenase in a soybean cell culture. Plant Physiol. 95:258-263.

Bhatt,-D.; Parrott,-W.A.; Collins,-G.B.; Hildebrand,-D.F. 1991. Agrobacterium induced gall formation in lipoxygenase mutant isolines of soybeans. Plant Cell Rep. 9:651-654.

Blahovec,-J. 1991. Use of denatured radioalbumin for determination of trypsin and chymotrypsin inhibitors in different plant seeds. J. Agric. Food Chem. 39:276-279.

Boerma,-H.R.; Hussey,-R.S.; Phillips,-D.V.; Wood,-E.D.; Finnerty,-S.L. 1991. Registration of 'Bryan' soybean. Crop Sci. 31:487.

Boki,-K.; Wada,-T.; Ohno,-S. 1991. Effects of filtration through activated carbons on peroxide, thiobarbituric acid and carbonyl values of autoxidized soybean oil. J. Am. Oil Chem. Soc. 68:561-565.

Bowers, G.R., Jr.; Goodman, R.M. 1991. Strain specificity of soybean mosaic virus seed transmission in soybean. Crop Sci. 31:1171-1174.

Bradner,-N.R.; Simpson,-R.D.; Hadlock,-A.A. 1991. PS 42 soybean. Can. J. Plant Sci. Rev. Can. Phytotech. 71:177-178.

Bruni,-F.; Leopold,-A.C. 1991. Glass transitions in soybean seed. Relevance to anhydrous biology. Plant Physiol. 96:660-663.

Caetano-Anolles,-G.; Bassam,-B.J.; Gresshoff,-P.M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technol. 9:553-557.

Callaci,-J.J.; Smarrelli,-J.-Jr. 1991. Regulation of the inducible nitrate reductase isoform from soybeans. Biochim. Biophys. Acta Int. J. Biochem. Biophys. 1088:127-130.

Carpenter,-A.S.; Lewis,-S.A. 1991. Aggressiveness and reproduction of four Meloidogyne arenaria populations on soybean. J. Nematol. 23:232-238.

Carroll,-K.K. 1991. Review of clinical studies on cholesterol-lowering response to soy protein. J. Am. Diet. Assoc. 91:820-827.

Carson,-M.L.; Arnold,-W.E.; Todt,-P.E. 1991. Predisposition of soybean seedlings to fusarium root rot with trifluralin. Plant Dis. 75:342-347.

Caulfield,-F.; Bunce,-J.A. 1991. Influence of the environment during seed development on the morphology and growth rate of soybean seedlings. Bot. Gaz. 152:59-63.

Caviness,-C.E. 1991. Registration of soybean germplasm lines R85-395 and R88-1259, with quintafoliolate leaves. Crop Sci. 31:495-496.

Caviness,-C.E.; Riggs,-R.D.; Rupe,-J.C. 1991. 'Narow M'--a metribuzin-tolerant soybean cultivar. Arkansas Farm Res. Arkansas Agric. Exp. Stn. 40:10.

Chamovitz,-D.; Pecker,-I.; Hirschberg,-J. 1991. The molecular basis of resistance to the herbicide norflurazon. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:967-974.

Chen,-P.; Buss,-G.R.; Roane,-C.W.; Tolin,-S.A. 1991. Allelism among genes for resistance to soybean mosaic virus in strain-differential soybean cultivars. Crop Sci. 31:305-309.

Cheong,-J.J.; Hahn,-M.G. 1991. A specific, high-affinity binding site for the hepta-beta-glucoside elicitor exists in soybean membranes. Plant Cell 3:137-147.

Cheong,-J.J.; Birberg,-W.; Fugedi,-P.; Pilotti,-A.; Garegg,-P.J.; Hong,-N.; Ogawa,-T.; Hahn,-M.G. 1991. Structure-activity relationships of oligo-beta-glucoside elicitors of phytoalexin accumulation in soybean. Plant Cell 3:127-136.

Cho,-M.J.; Harper,-J.E. 1991. Effect of inoculation and nitrogen on isoflavonoid concentration in wild-type and nodulation-mutant soybean roots. Plant Physiol. 95:435-441.

Cho,-M.J.; Harper,-J.E. 1991. Effect of localized nitrate application on isoflavonoid concentration and nodulation in split-root systems of wild-type and nodulation-mutant soybean plants. Plant Physiol. 95:1106-1112.

Cianzio,-S.R.; Shultz,-S.P.; Fehr,-W.R.; Tachibana,-H. 1991. Registration of 'Archer' soybean. Crop Sci. 31:1707.

Cianzio,-S.R.; Green,-D.E; Chang,-C.S.; Shibles,-R.M. 1991. Developmental periods in soybean: photoperiod-sensitive X insensitive crosses evaluated at diverse latitudes. Crop Sci. 31:8-13.

Cianzio,-S.R.; Tachibana,-H. Mansur,-L.M.; Fehr,-W.R., Niblack,-T.L.; Schultz,-S.P.; Ruff,-R. Bidne,-K. 1991. Registration of A20 soybean germplasm resistant to brown stem rot, soybean cyst nematode, and iron deficiency chlorosis. Crop Sci. 31:1713-1714.

Clark,-D.B. 1991. Protein--from vegetables. Sch. Foodserv. J. 45:54-55.

Clark,-P.K.; Snyder,-H.E. 1991. Hydroperoxide formation in soybean seeds during storage. J. Am. Oil Chem Soc. 68:346-347.

Cloud,-G.L.; Rupe,-J.C. 1991. Morphological instability on a chlorate medium of isolates of Macrophomina phaseolin from soybean and sorghum. Phytopathology 81:892-895.

Coale,-F.J.; Grove,-J.H. 1991. Potassium utilization by no-till full-season and double-crop soybean. Agron. J. 83:190-194.

Cooper,-R.L.; Fausey,-N.R.; Streeter,-J.G. 1991. Yield potential of soybean grown under a subirrigation/drainage water management system. Agron. J. 83:884-887.

Cooper,-R.L.; Martin,-R.J.; McBlain,-B.A.; Fioritoo,-R.J.; St.-Martin,-S.K.; Calip-DuBois,-A.; Schmitthenner,-A.F. 1991. Registration of 'Hoyt' soybean. Crop Sci. 31:231-232.

Cooper,-R.L.; Martin,-R.J.; Walker,-A.K.; Schmitthenner,-A.F. 1991. Registration of 'Hobbit' soybean. Crop Sci. 31:231.

Cooper,-R.L.; Martin,-R.J.; Schmitthenner,-A.F.; McBlain,-B.A.; Fioritto,-R.J.; St. Martin,-S.K.; Calip-DuBois,-A. 1991. Registration of 'Hobbit 87' soybean. Crop Sci. 31:1093.

Cooper,-R.L.; Martin,-R.J.; Schmitthenner,-A.F.; McBlain,-B.A.; Fioritto,-R.J.; St. Martin,-S.K.; Calip-DuBois,-A. 1991. Registration of 'Sprite 87' soybean. Crop Sci. 31:1093.

Cooper,-R.L.; Martin,-R.J.; Schmitthenner,-A.F.; McBlain,-B.A.; Fioritto,-R.J.; St. Martin,-S.K.; Calip-DuBois,-A. 1991. Registration of 'Amcor 89' soybean. Crop Sci. 31:1094.

Covey,-J.E.; Wan,-P.J. 1991. Hydrogenation of oxidized soybean oil. J. Am. Oil Chem. Soc. 68:337-338.

Creelman,-R.A.; Mullet,-J.E. 1991. Abscisic acid accumulates at positive turgor potential in excised soybean seedling growing zones. Plant Physiol. 95:1209-1213.

Crookston,-R.K.; Kurle,-J.E.; Copeland,-P.J.; Ford,-J.H.; Lueschen,-W.E. 1991. Rotational cropping sequence affects yield of corn and soybean. Agron. J. 83:108-113.

Crowell,-D.N.; Amasino,-R.M. 1991. Induction of specific mRNAs in cultured soybean cells during cytokinin or auxin starvation. Plant Physiol. Rockville, Md.: American Society of Plant Physiologists. 95:711-715.

Cucurou,-C.; Battioni,-J.P.; Daniel,-R.; Mansuy,-D. 1991. Peroxidase-like activity of lipoxygenases: different substrate specificity of potato 5-lipoxygenase and soybean 15-lipoxygenase and particular affinity of vitamin E derivatives for the 5-lipoxygenase. Biochim. Biophys. Acta Int. J. Biochem. Biophys. 1081:99-105.

Cunningham,-S.; Kollmeyer,-W.D.; Stacey,-G. 1991. Chemical control of interstrain competition for soybean nodulation by Bradyrhizobium japonicum. Appl. Environ. Microbiol. 57:1886-1892.

Dakora,-F.D.; Appleby,-C.A.; Atkins,-C.A. 1991. Effect of pO2 on the formation and status of leghemoglobin in nodules of cowpea and soybean. Plant Physiol. 95:723-730.

De-Cianzio,-S.R.; Green,-D.E.; Chang,-C.S.; Shibles,-R.M. 1991. Developmental periods in soybean: photoperiod-sensitive X insensitive crosses evaluated at diverse latitudes. Crop Sci. 31:8-13.

Denison,-R.F.; Smith,-D.L.; Legros,-T.; Layzell,-D.B. 1991. Noninvasive measurement of internal oxygen concentration of field-grown soybean nodules. Agron. J. 83:166-169.

Devine,-T.E.; Kilen,-T.C.; O'Neill,-J.J. 1991. Genetic linkage of the phytopthora resistance gene Rps2 and the nodulation response gene Rj2 in soybean. Crop Sci. 31:713-715.

Devine,-T.E.; Kuykendall,-L.D.; O'Neill,-J.J. 1991. Nodulation interaction of the soybean allele Rj2 with Asiatic isolates of Bradyrhizobium japonicum. Crop Sci. 31:1129-1131.

Diers,-B.W.; Voss,-B.K.; Fehr,-W.R. 1991. Moving-mean analysis of field tests for iron efficiency of soybean. Crop Sci. 31:54-56.

Djekoun,-A.; Planchon,-C. 1991. Water status effect on dinitrogen fixation an photosynthesis in soybean. Agron. J. 83:316-322.

Edwards,-D.R.; Ferguson,-J.A.; Fryar,-E.O. 1991. Assessing feasibility of off-stream reservoirs for soybean production in eastern Arkansas. Arkansas Farm Res. Arkansas Agric. Exp. Stn. 40:6-7.

Egli,-D.B.; Zhen-wen,-Y. 1991. Crop growth rate and seeds per unit area in soybean. Crop Sci. 31:439-442.

Elden,-T.C. 1991. Influence of photoperiod and temperature on the reproductive diapause of two different geographic populations of the Mexican bean beetle (Coleoptera: Coccinellidae) and their cross progeny. J. Entomol. Sci. 26:119-121.

Eskridge,-K.M.; Johnson,-B.E. 1991. Expected utility maximization and selection of stable plant cultivars. Theor. Appl. Genet. 81:825-832.

Estabrook,-E.M.; Sengupta-Gopalan,-C. 1991. Differential expression of phenylalanine ammonia-lyase and chalcone synthase during soybean nodule development. Plant Cell 3:299-308.

Fehr,-W.R.; Welke,-G.A.; Hammond,-E.G.; Duvick,-D.N.; Cianzio,-S.R. 1991. Inheritance of reduced palmitic acid content in seed oil of soybean. Crop Sci. 31:88-89.

Fehr,-W.R.; Welke,-G.A.; Hammond,-E.G. Duvick,-D.N., Cianzio,-S.R. 1991. Inheritance of elevated palmitic acid content in soybean seed oil. Crop Sci. 31:1522-1524.

Felland,-C.M.; Pitre,-H.N. 1991. Diversity and density of foliage-inhabiting arthropods in irrigated and dryland soybean Mississippi. Environ. Entomol. 20:498-506.

Fernandez-Pinto,-V.E.; Vaamonde,-G.; Brizzio,-S.B.; Apro,-N. 1991. Aflatoxin production in soybean varieties grown in Argentina. J. Food Prot. 54:542-545.

Frank,-R.L.; Vodkin,-L.O. 1991. Sequence and structure of a phenylalanine ammonia-lyase gene from Glycine max. DNA Sequence J. DNA Sequencing Mapp. 1:335-346.

Friedman,-M.; Brandon,-D.L.; Bates,-A.H.; Hymowitz,-T. 1991. Effect of heat on the nutritional quality and safety of soybean cultivars. In: Nutritional and toxicological consequences of food processing. M. Friedman (ed.) Plenum Publishing Corp. N.Y. pp. 339-363.

Friedman,-M.; Brandon,-D.L.; Bates,-A.H.; Hymowitz,-T. 1991. Comparison of a commercial soybean cultivar and an isoline lacking the Kunitz trypsin inhibitor: composition, nutritional value, and effects of heating. J. Agric. Food Chem. 39:327-335.

Fujiwara,-T.; Naito,-S.; Chino,-M.; Nagata,-T. 1991. Electroporated protoplasts express seed specific gene promoters. Plant Cell Rep. 9:602-606.

Garrel,-D.R.; Verdy,-M.; PetitClerc,-C.; Martin,-C.; Brule,-D.; Hamet,-P. 1991. Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. Am. J. Clin. Nutr. 53:665-669.

Gee,-M.A.; Hagen,-G.; Guilfoyle,-T.J. 1991. Tissue-specific and organ-specific expression of soybean auxin-responsive transcripts GH3 and SAURs. Plant Cell. 3:419-430.

Gildersleeve,-R.R; Smith,-G.R.; Pemberton,-I.J.; Gilbert,-C.L. 1991. Detection of isoflavones in seedling subterranean clover. Crop Sci. 31:889-892.

Gillikin,-J.W.; Graham,-J.S. 1991. Purification and developmental analysis of the major anionic peroxidase from the seed coat of Glycine max. Plant Physiol. 96:214-220.

Godwin,-I.; Todd,-G.; Ford-Lloyd,-B.; Newbury,-H.J. 1991. The effects of acetosyringone and pH on Agrobacterium-mediated transformation vary according to plant species. Plant Cell Rep. 9:671-675.

Gojon,-A.; Wakrim,-R.; Passama,-L.; Robin,-P. 1991. Regulation of NO3- assimilation by anion availability in excised soybean leaves. Plant Physiol. 96:398-405.

Goodrick, -B.J.; Kuhn, -C.W.; Hussey, -R.S. 1991. Restricted systemic movement of cowpea chlorotic mottle virus in soybean with nonnecrotic resistance. Phytopathology 81:1426-1431.

Goodyear,-S.N.; Ormrod,-D.P. 1991. Within-photoperiod differences in ozone sensitivity of soybean and tomato. Can. J. Plant Sci. Rev. Can. Phytotech. Ottawa: Agricultural Institute of Canada. 71:269-278.

Grabau,-L.J.; Pearce,-R.C.; Konsler,-J.V. 1991. Influence of ethephon on lowest pod height and yield of soybean. Agron. J. 83:175-177.

Graham,-T.L. 1991. Flavonoid and isoflavonoid distribution in developing soybean seedling tissues and in seed and root exudates. Plant Physiol. 95:594-603.

Gray,-S.M.; Power,-A.G.; Smith,-D.M.; Seaman,-A.J.; Altman,-N.S. 1991. Aphid transmission of barley yellow dwarf virus: Acquisition access periods and virus concentration requirements. Phytopathology 81:535-545.

Grayburn,-W.S.; Schneider,-G.R.; Hamilton-Kemp,-T.R.; Bookjans,-G.; Ali,-K.; Hildebrand,-D.F. 1991. Soybean leaves contain multiple lipoxygenases. Plant Physiol. 95:1214-1218.

Griffin,-J.L.; Dunand,-R.T.; Baker,-J.B.; Regan,-R.P.; Cohn,-M.A. 1991. Integrating red rice control measures in soybean-rice rotations. La. Agric. La. Agric. Exp. Stn. 34:6-7.

Griffor,-M.C.; Vodkin,-L.O.; Singh,-R.J.; Hymowitz,-T. 1991. Fluorescent in situ hybridization to soybean metaphase chromosomes. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 17:101-109.

Groose,-R.W.; Palmer,-R.G. 1991. Gene action governing anthocyanin pigmentation in soybean. J. Hered. 82:498-501.

Guiamet,-J.J.; Schwartz,-E.; Pichersky,-E.; Nooden,-L.D. 1991. Characterization of cytoplasmic and nuclear mutations affecting chlorophyll and chlorophyll-binding proteins during senescence in soybean. Plant Physiol. 96:227-231.

Gunderson,-C.A.; Taylor,-G.E.-Jr. 1991. Ethylene directly inhibits foliar gas exchange in Glycine max. Plant Physiol. 95:337-339.

Gupta,-G.; Sandhu,-R.; Mulchi,-C. 1991. Changes in foliar concentration of abscisic acid in soybean in response to sulfur dioxide fumigation. J. Environ. Qual. 20:151-152.

Hallmark,-W.B.; Morris,-H.F.; Brown,-L.P.; Wall,-D.A. 1991. SOY-DRIS, a user-friendly computer software program that evaluates nutritional needs of soybeans. La. Agric. La. Agric. Exp. Stn. 34:8-9.

Hamer,-J. 1991. Know your soybean insects. Inf. Sheet Miss. Agric. For. Exp. Stn. (225) 2:ill.

Hammatt,-N.; Blackhall,-N.W.; Davey,-M.R. 1991. Variation in the DNA content of glycine species. J. Exp. Bot. 42:659-665.

Han,-Y.; Parsons,-C.M.; Hymowitz,-T. 1991. Nutritional evaluation of soybeans varying in trpysin inhibitor content. Poultry Sci. 70:896-906.

Han,-I.S.; Jongewaard,-I.; Fosket,-D.E. 1991. Limited expression of a diverged beta-tubulin gene during soybean (Glycine max [L.] Merr.) development. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:225-234.

Hanson,-W.D. 1991. Root characteristics associated with divergent selection for seedling aluminum tolerance in soybean. Crop Sci. 31:125-129.

Hanson,-W.D. 1991. Seed protein content and delivery of assimilates to soybean seed embryos. Crop Sci. 31:1600-1604.

Harrison,-M.J.; Lawton,-M.A.; Lamb,-C.J.; Dixon,-R.A. 1991. Characterization of a nuclear protein that binds to three elements within the silencer region of a bean chalcone synthase gene promoter. Proc. Natl. Acad. Sci. USA 88:2515-2519. ill.

Hart,-S.E.; Glenn, -S.; Kenworthy,-W.W. 1991. Tolerance and the basis for selectivity to a 2,4-D in perennial Glycine species. Weed Sci. 39:535-539.

Hartwig,-E.E.; Kilen,-T.C. 1991. Yield and composition of soybean seed from parents with different protein, similar yield. Crop Sci. 31:290-292.

Hartwig,-E.E.; Jones,-W.F.; Kilen,-T.C. 1991. Identification and inheritance of inefficient zinc absorption in soybean. Crop Sci. 31:61-63.

Heaney,-R.P.; Weaver,-C.M.; Fitzsimmons,-M.L. 1991. Soybean phytate content: effect on calcium absorption. Am. J. Clin. Nutr. 53:745-747.

Heatherly,-L.G.; Young,-L.D. 1991. Soybean and soybean cyst nematode response to soil water content in loam and clay soils. Crop Sci. 31:191-196.

Heatherly,-L.G.; Pringle,-H.C.-III 1991. Soybean cultivars' response to flood irrigation of clay soil. Agron. J. 83:231-236.

Hedges,-B.R.; Palmer,-R.G. 1991. Tests of linkage of isozyme loci with five primary trisomics in soybean, Glycine max (L.) Merr. J. Hered. 82:494-496.

Herbert,-D.A.; Zehnder,-G.W.; Day,-E.R. 1991. Evaluation of a pest advisory for corn earworm (Lepidoptera: Noctuidae) infestations in soybean. J. Econ. Entomol. 84:515-519.

Herman,-M.; Hussey,-R.S.; Boerma,-H.R. 1991. Penetration and development of Meloidogyne incognita on roots of resistant soybean genotypes. J. Nematol. 23:155-161.

Higley,-L.G.; Pedigo,-L.P. 1991. Soybean yield responses and intraspecific competition from simulated seedcorn maggot injury. Agron. J. 83:135-139.

Hymowitz,-T. 1991. Kunitz soybean variety. Illinois Research 33 (no 1/2):8-9.

Hymowitz,T.; Palmer, R.G.; Singh, R.J. 1991. Cytogenetics of the genus Glycine. In: T.Tsuchiya and P.K. Gupta (eds.) Chromosome engineering in Plants: genetics, breeding, evolution, part B. Elsevier Science Publishers, Netherlands. pp 53-63.

Hymowitz,-T.; Bernard,-R.L. 1991. Origin of the soybean and germplasm introduction, and development in North America. In H.L. Shands (ed.). Use of plant introductions in cultivar development, part 1 CSSA Spec. Pub. 17. 149-167.

Imsande,-J. 1991. Regulation of nodule efficiency by the undisturbed soybean plant. J. Exp. Bot. 42 (238):687-691.

Inamdar,-N.M.; Ehrlich,-K.C.; Ehrlich,-M. 1991. CpG methylation inhibits binding of several sequence-specific DNA-binding proteins from pea, wheat, soybean and cauliflower. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 17:111-123.

Iwahashi,-H.; Albro,-P.W.; McGown,-S.R.; Tomer,-K.B.; Mason,-R.P. 1991. Isolation and identification of alpha-(4-Pyridyl-1-oxide)-N-tert-butylnitrone radical adducts formed by the decomposition of the hydroperoxides of linoleic acid, linolenic acid, and arachidonic acid by soybean lipoxygenase. Arch. Biochem. Biophys. 285:172-180.

James,-E.K.; Sprent,-J.I.; Minchin,-F.R.; Brewin,-N.J. 1991. Intercellular location of glycoprotein in soybean nodules: effect of altered rhizosphere oxygen concentration. Plant Cell Environ. 14:467-476.

Jayaram,-C.; Hill,-J.H.; Miller,-W.A. 1991. Nucleotide sequences of the coat protein genes of two aphid-transmissible strains of soybean mosaic virus. J. Gen. Virol. 72 (pt.4):1001-1003.

Ji,-L.; Wood,-S.; Becana,-M.; Klucas,-R.V. 1991. Purification and characterization of soybean root nodule ferric leghemoglobin reductase. Plant Physiol. 96:32-37.

Jideani,-I.A.O.; Okeke,-C.R. 1991. Comparative study of microorganisms and sensory attributes of condiments from the fermentation of different seeds. Plant Food Hum. Nutr. 41:27-34.

Jouanneau,-J.P.; Lapous,-D.; Guern,-J. 1991. In plant protoplasts, the spontaneous expression of defense reactions and the responsiveness to exogenous elicitors are under auxin control. Plant Physiol. 96:459-466.

Kao,-W.Y.; Forseth,-I.N. 1991. The effects of nitrogen, light and water availability on tropic leaf movements in soybean (Glycine max). Plant Cell Environ. 14:287-293.

Karel,-A.K.; Ashimogo,-G.C. 1991. Economics of insect control on common beans and soybeans in Tanzania. J. Econ. Entomol. 84:996-1000.

Kawashima,-I.; Inokuchi,-Y.; Chino,-M.; Kimura,-M.; Shimizu,-N. 1991. Isolation of a gene for a metallothionein-like protein from soybean. Plant Cell Physiol. 32:913-916.

Keen,-N.T.; Buzzell,-R.I. 1991. New disease resistance genes in soybean against Pseudomonas syringae pv glycinea: evidence that one of them interacts with a bacterial elicitor. Theor. Appl. Genet. 81:133-138.

Kiang,-Y.T.; Bult,-C.J. 1991. Genetic and linkage analysis of aconitate hydratase variants in soybean. Crop Sci. 31:322-325.

Kilen,-T.C. 1991. Genetic resources and utilization of the USDA soybean germplasm collection pp. 30-37. In: R.F. Wilson (ed.) Designing value-added soybeans for markets of the future. Amer. Oil Chem. Soc.

Kim,-Y.S.; Chae,-H.Z. 1991. Purification and properties of malonyl-CoA synthetase from Rhizobium japonicum. Biochem. J. 273 (pt.3):511-516.

King,-B.J.; Layzell,-D.B. 1991. Effect of increases in oxygen concentration during the argon-induced decline in nitrogenase activity in root nodules of soybean. Plant Physiol. 96:376-381.

Klepper,-L. 1991. NOx evolution by soybean leaves treated with salicylic acid and selected derivatives. Pestic. Biochem. Physiol. 39:43-48.

Klubertanz,-T.H.; Pedigo,-L.P.; Carlson,-R.E. 1991. Impact of fungal epizootics on the biology and management of the twospotted spider mite (Acari: Tetranychidae) in soybean. Environ. Entomol. Lanham, Md.: Entomological Society of America. 20:731-735.

Klucas,-R.V.; Appleby,-C.A. 1991. Nicotinate, nicotinamide, and the reactivity of leghemoglobin in soybean root nodules. Plant Physiol. 95:551-555.

Koening,-S.R.; Anand,-S.C. 1991. Effects of wheat and soybean planting date on Heterodera glycines population dynamics and soybean yield with conventional tillage. Plant Dis. 75:301-304.

Komatsuda,-T.; Kaneko,-K.; Oka,-S. 1991. Genotype X sucrose interactions for somatic embryogenesis in soybean. Crop Sci. 31:333-337.

Kuang,-A.; Peterson,-C.M.; Dute,-R.R. 1991. Changes in soybean raceme and petiole anatomy induced by 6-benzylaminopurine. Ann. Bot. 67:23-27.

Kulik,-M.M.; Yaklich,-R.W. 1991. Soybean seed coat structures: relationship to weathering resistance and infection by the fungus Phomopsis phaseoli. Crop Sci. 31:108-113.

La-Favre,-A.K.; Sinclair,-M.J.; La-Favre,-J.S.; Eaglesham,-A.R.J. 1991. Bradyrhizobium japonicum native to tropical soils: novel sources of strains for inoculants for US-type soya bean. Trop. Agric. 68:243-248.

Lambert,-L.; Heatherly,-L.G. 1991. Soil water potential:effects on soybean looper feeding on soybean leaves. Crop Sci. 31:1625-1628.

Laszlo,-J.A. 1991. Changes in endogenous and exogenous iron-reducing capability of soybean hull during development. Cereal Chem. 68:21-24.

Lawton,-M.A.; Dean,-S.M.; Dron,-M.; Kooter,-J.M.; Kragh,-K.M.; Harrison,-M.J.; Yu,-L.; Tanguary,-L.; Dixon,-R.A.; Lamb,-C.J. 1991. Silencer region of a chalcone synthase promoter contains multiple binding sites for a factor, SBF-1, closely related to GT-1. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:235-249.

Lee,-H.; Chung,-B.H.; Park,-Y.H. 1991. Concentration of tocopherols from soybean sludge by supercritical carbon dioxide. J. Am. Oil Chem. Soc. 68:571-573.

Lessard,-P.A.; Allen,-R.D.; Bernier,-F.; Crispino,-J.D.; Fujiwara,-T.; Beachy,-R.N. 1991. Multiple nuclear factors interact with upstream sequences of differentially regulated beta-conglycinin genes. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:397-413.

Lindstrom,-J.T.; Vodkin,-L.O. 1991. A soybean cell wall protein is affected by seed color genotype. Plant Cell. 3:561-571.

Likimani,-T.A.; Sofos,-J.N.; Maga,-J.A.; Harper,-J.M. 1991. Extrusion cooking of corn/soybean mix in presence of thermostable alpha-amylase. J. Food Sci. Off. Publ. Inst. Food Technol. 56:99-105, 108.

Liljebjelke,-K.A.; Franceschi,-V.R. 1991. Differentiation of mesophyll and paraveinal mesophyll in soybean leaf. Bot. Gaz. 152:34-41.

Lin,-L.S.; Yuen,-H.K.; Varner,-J.E. 1991. Differential scanning calorimetry of plant cell walls. Proc. Natl. Acad. Sci. USA 88:2241-2243.

Liu,-Z.Q.; Fer,-A.; Lecocq,-F.M. 1991. Imazaquin; a promising herbicide for the control of dodder (Cuscuta spp.) in soybean (Glycine max). L'imazaquine: un herbicide prometteur pour la lutte curative contre la cuscute (Cuscuta ssp.) dans les cultures de soja (Glycine max). Weed Res. 31:33-40.

Loper,-M.T.; Spanswick,-R.M. 1991. Auxin transport in suspension-cultured soybean root cells. II. Anion effects on carrier-mediated uptake. Plant Physiol. 96:192-197.

Loper,-M.T.; Spanswick,-R.M. 1991. Auxin transport in suspension-cultured soybean root cells. I. Characterization. Plant Physiol. 96:184-191.

Louie,-R.; Findley,-W.R.; Knoke,-J.K.; McMullen,-M.D. 1991. Genetic basis of resistance in maize to five maize dwarf mosaic virus strains. Crop Sci. 31:14-18.

McBlain,-B.A.; Zimmerly,-M.M.; Schmitthenner,-A.F.; Hacker,-J.K. 1991. Tolerance to phytophthora rot in soybean: I. Studies of the cross 'Ripley' X 'Harper'. Crop Sci. 31:1405-1411.

McBlain,-B.A.; Hacker,-J.K.; Zimmerly,-M.M.; Schmitthenner,-A.F. 1991. Tolerance to phytophthora rot in soybean: II. Evaluation of three tolerance screening methods. Crop Sci. 31:1412-1417.

McBlain,-B.A.; Fjoritto,-R.J.; St-Martin,-S.K.; Calip-Dubois,-A.; Schmitthenner,-A.F.; Cooper,-R.L.; Martin,-R.J. 1991. Registration of 'Edison' soybean. Crop Sci. 31:488-489.

McBlain,-B.A.; Fioritto,-R.J.; St-Martin,-S.K.; Calip-DuBois,-A.; Schmitthenner,-A.F.; Cooper,-R.L.; Martin,-R.J. 1991. Registration of 'Hayes' soybean. Crop Sci. 31:488.

McBlain,-B.A.; Fioritto,-R.J.; St-Martin,-S.K.; Calip-DuBois,-A.; Schmitthenner,-A.F.; Cooper,-R.L.; Martin,-R.J. 1991. Registration of 'Chapman' soybean. Crop Sci. 31:487-488.

McBlain,-B.A.; Schmitthenner,-A.F. 1991. Evaluations of recurrent selection for Phytophthora tolerance. Res. Bull. Ohio Agric. Res. Dev. Cent. (1187) 29 p.

McSorley,-R.; Dickson,-D.W. 1991. Determining consistency of spatial dispersion of nematodes in small plots. J. Nematol. 23:65-72.

Mancusco, N.; Caviness,-C.E. 1991. Association of selected plant traits wtih lodging of four determinate soybean cultivers. Crop Sci. 31:911-914.

Mason,-L.J.; Pashley,-D.P. 1991. Sperm competition in the soybean looper (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 84:268-271.

Mengistu,-A.; Tachibana,-H.; Grau,-C.R. 1991. Selective medium for isolation and enumeration of Phialophora gregata from soybean straw and soil. Plant Dis. 75:196-199.

Mernaugh,-R.L.; Hill,-J.H.; Durand,-D.P.; Van-Deusen,-R.A.; Harris,-K.F. 1991. Properties and potential applications of syngenetic monoclonal anti-idiotypic antibodies: use as a reference standard in enzyme-linked immunosorbent assay. Phytopathology 81:274-282.

Messina,-M.; Messina,-V. 1991. Increasing use of soyfoods and their potential role in cancer prevention. J. Am. Diet. Assoc. 91:836-840.

Miao,-G.H.; Hirel,-B.; Marsolier,-M.C.; Ridge,-R.W.; Verma,-D.P.S. 1991. Ammonia-regulated expression of a soybean gene encoding cytosolic glutamine synthetase in transgenic Lotus corniculatus. Plant Cell 3:11-22.

Mir,-P.S.; Burton,-J.H.; Buchanan-Smith,-J.G. 1991. Nutritional performance of calves fed milk replacers containing processed soybean products. Can. J. Anim. Sci. 71:97-106.

Mohamed,-A.I.; Mebrahtu,-T.; Rangappa,-M. 1991. Nutrient composition and anti-nutritional factors in selected vegetable soybean (Glycine max [L.] Merr.). Plant Food Hum. Nutr. 41:89-100.

Morandi,-D.; Le-Quere,-J.L. 1991. Influence of nitrogen on accumulation of isosojagol (a newly detected coumestan in soybean) and associated isoflavonoids in roots and nodules of mycorrhizal and non-mycorrhizal soybean. New Phytol. 117:75-79.

Moore,-S.H. 1991. Uniformity of plant spacing effect on soybean population parameters. Crop Sci. 31:1049-1051.

Moore,-S.H.; Hartwig,-E.E.; Robertson-C.A.-III. 1991. Increasing soybean yields at early and late planting dates by delayed flowering. La. Agric. La. Agric. Exp. Stn. 34:4-5.

Moorman,-T.B.; Dowler,-C.C. 1991. Herbicide and rotation effects on soil and rhizosphere microorganisms and crop yields. Agric. Ecosyst. Environ. 35:311-325.

Mpepereki,-S.; Wollum,-A.G.-II. 1991. Diversity of indigenous Bradyrhizobium japonicum in North Carolina soils. Biol. Fertil. Soils. 11:121-127.

Muehlbauer,-G.J.; Staswick,-P.E.; Specht,-J.E.; Graef,-G.L.; Shoemaker,-R.C.; Keim,-P. 1991. RFLP mapping using near-isogenic lines in the soybean [Glycine max (L.) Merr.]. Theor. Appl. Genet. 81:189-198.

Newman,-K.D.; VanToai,-T.T. 1991. Developmental regulation and organ-specific expression of soybean alcohol dehydrogenase. Crop Sci. 31:1253-1257.

Nickell,-A.D.; Wilcox,-J.R.; Cavins,-J.F. 1991. Genetic relationships between loci controlling palmitic and linolenic acids in soybean. Crop Sci. 31:11369-1171.

Nieh,-C.D.; Snyder,-H.E. 1991. Solvent extraction of oil from soybean flour. II. Pilot plant and two-solvent extractions. J. Am. Oil Chem. Soc. 68:250-253.

Nieh,-C.D.; Snyder,-H.E. 1991. Solvent extraction of oil from soybean flour. I. Extraction rate, a countercurrent extraction system, and oil quality. J. Am. Oil Chem. Soc. 68:246-249.

Oliver,-L.R.; Chandler,-J.M.; Buchanan,-G.A. 1991. Influence of geographic region jimsonweed (Datura stramonium) interference in soybeans (Glycine max) and cotton (gossypium hirsutum). Weed Sci. 39:585-589.

Opitz,-A. 1991. School food service sings soy's praises. Sch. Foodserv. J. 45:56-57.

Orf,-J.H.; Schaus,-P.J.; Kennedy,-B.W. 1991. Registration of 'Minnatto' soybean. Crop Sci. 31:233.

Orf,-J.H.; Lambert,-J.W.; Kennedy,-B.W. 1991. Registration of 'Sturdy' soybean. Crop Sci. 31:486-481.

Orf,-J.H.; Lambert,-J.W.; Kennedy,-B.W. 1991. Registration of 'Proto' soybean. Crop Sci.31:486.

Orf,-J.H.; Lambert,-J.W.; Kennedy,-B.W. 1991. Registration of 'Kato' soybean. Crop Sci. 31:233.

Orf,-J.H.; Kennedy,-B.W.; Schaus,-P.J. 1991. Registration of 'Kasota' soybean. Crop Sci. 31:1092.

Ovenell,-K.H.; Lusby,-K.S.; Horn,-G.W.; McNew,-R.W. 1991. Effects of lactational status on forage intake, digestibility, and particulate passage rate of beef cows supplemented with soybean meal, wheat middlings, and corn and soybean meal. J. Anim. Sci. 69:2617-2623.

Owen,-F.G.; Larson,-L.L. 1991. Corn distillers dried grains versus soybean meal in lactation diets. J. Dairy Sci. 74:972-979.

Palaniappan,-S.; Proctor,-A. 1991. Evaluation of soy oil lutein isotherms obtained with selected adsorbents in hexane miscellas. J. Am. Oil Chem. Soc. 68:79-82.

Park,-K.H.; Cho,-Y.D. 1991. Purification of monomeric agmatine iminohydrolase from soybean. Biochem. Biophys. Res. Commun. 174:32-36.

Pennazio,-S.; Roggero,-P. 1991. Rapid ethylene production in soybean in response to the Cupric ion. Ann. Bot. 67:247-249.

Perry,-M.C.; McIntosh,-M.S.. 1991. Geographical patterns of variation in the USDA soybean germplasm collection: I. Morphological traits. Crop Sci. 31:1350-1355.

Perry,-M.C.; McIntosh,-M.S.; Stoner,-A.K. 1991. Geographical pattern of variation in the USDA soybean germplasm collection: II. Allozyme frequencies. Crop Sci. 1356-1360.

Pfeiffer,-T.W.; Suryati,-D.; Egli,-D.B. 1991. Soybean plant introductions selected for seed filling period or yield: Performace as parents. Crop Sci. 31:1418-1421.

Phillips,-A.J.L.Variation in virulence to dry beans, soybeans and maize among isolates of Rhizoctonia solani from beans. Ann. Appl. Biol. Warwick: Association of Applied Biologists. 118:9-17.

Pick,-D.H.; Park,-T.A. 1991. The competitive structure of U.S. agricultural exports. Am. J. Agric. Econ. 73:133-141.

Prior,-S.A.; Rogers,-H.H.; Sionit,-N.; Patterson,-R.P. 1991. Effects of elevated atmospheric CO2 on water relations of soya bean. Agric. Ecosyst. Environ. 35:13-25.

Rennie,-B.D.; Tanner,-J.W. 1991. New alleles at the Fan locus in the soybean line A5. Crop Sci. 31:297-301.

Rennie,-B.D.; Tanner,-J.W. 1991. Fatty acid composition of oil from soybean leaves grown at extreme temperatures. J. Am. Oil Chem. Soc. 68:106-108.

Riggs,-R.D.; Schmitt,-D.P. 1991. Optimization of the Heterodera glycines race test procedure. J. Nematol. 23:149-154.

Roberts,-J.K.; Key,-J.L. 1991. Isolation and characterization of a soybean hsp70 gene. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:671-683. ill.

Rols,-J.L.; Goma,-G. 1991. Enhanced oxygen transfer rates in fermentation using soybean oil-in-water dispersions. Biotechnol. Lett. 13:7-12.

Rosa,-L.M.; Dillenburg,-L.R.; Forseth,-I.N. 1991. Responses of soybean leaf angle, photosynthesis and stomatal conductance to leaf and soil water potential. Ann. Bot. 67:51-58.

Rose,-I.A.; Colton,-R.T. 1991. Registration of Australian oilseed cultivars: Glycine max (L.) Merr. (soybean) cOxley, Reg. No. AROC 4-2/22, registered 5 September 1990. Aust. J. Exp. Agric. 31:138.

Rose,-I.A.; Colton,-R.T. 1991. Register of Australian oilseed cultivars: Glycine max (L.) Merr. (soybean) cIntrepid, Reg. No. AROC 4-2/23, Registered 5 September 1990. Aust. J. Exp. Agric. 31:137.

Rupe,-J.C.; Gbur,-E.E.; Marx,-D.M. 1991. Cultivar responses to sudden death syndrome of soybean. Plant Dis. 75:47-50.

Sangwan,-I.; O'Brian,-M.R. 1991. Evidence for an inter-organismic heme biosynthetic pathway in symbiotic soybean root nodules. Science 251 (4998):1220-1222.

Sadowsky,-M.J.; Cregan,-P.B.; Gottfert,-M.; Sharma,-A.; Gerhold,-D.; Rodriguez-Quinones,-F.; Keyser,-H.H.; Hennecke,-H.; Stacey,-G. 1991. The Bradyrhizobium japonicum nolA gene and its involvement in the genotype-specific nodulation of soybeans. Proc. Natl. Acad. Sci. USA 88:637-641.

Salminen,-S.O.; Streeter,-J.G. 1991. Improved analysis of metabolite uptake by Bradyrhizobium japonicum bacteroids. Can. J. Microbiol. 37:238-243.

Sanders,-L.C.; Wang,-C.S.; Walling,-L.L.; Lord,-E.M. 1991. A homolog of the substrate adhesion molecule vitronectin occurs in four species of flowering plants. Plant Cell. 3:629-635.

Schmidt,-A.; Datta,-K.; Marcus,-A. 1991. Peptidyl proline hydroxylation and the growth of a soybean cell culture. Plant Physiol. 96:656-659.

Schmitt,-D.P.; Riggs,-R.D. 1991. Influence of selected plant species on hatching of eggs and development of juveniles of Heterodera glycines. J. Nematol. 23:1-6.

Schnepf,-M.; Spencer,-G.; Carlat,-J. 1991. Chemical and sensory characteristics of stored menhaden oil/soybean oil blends. J. Am. Oil Chem. Soc. 68:281-284.

Seigmund,-K.D.; Plattner,-H.J.; Diekmann,-H. 1991. Purification of ferrichrome synthetase from Aspergillus quadricinctus and characterisation as a phosphopantetheine containing multienzyme complex. Biochem. Biophys. Acta Int. J. Biochem. Biophys. 1076:123-129.

Sharma,-S.K.; Mehta,-H. 1991. Manifestation of genetic diversity for physiological traits in soybean under two cropping systems. Trop. Agric. 68:202-206.

Shaw,-D.R.; Wixson,-M.B. 1991. Postemergence combinations of imazaquin or imazethapyr with AC 263,222 for weed control in soybean (Glycine max). Weed Sci. 39:644-649.

Shaw,-D.R.; Newsom,-L.J.; Smith,-C.A. 1991. Influence of cultivation timing on chemical control of sicklepod (Cassia obtusifolia) in soybean (Glycine max). Weed Sci. 39:67-72.

Sheehy,-J.E.; Webb,-J. 1991. Oxygen diffusion pathways and nitrogen fixation in legume root nodules. Ann. Bot. 67:85-91.

Shen,-C.F.; De-Man,-L.; Buzzell,-R.I.; De-Man,-J.M. 1991. Yield and quality of tofu as affected by soybean and soymilk characteristics: glucono-delta-lactone coagulant. J. Food Sci. Off. Publ. Inst. Food Technol. 56:109-112.

Shibata,-D.; Kato,-T.; Tanaka,-K. 1991. Nucleotide sequences of a soybean lipoxygenase gene and the short intergenic region between and upstream lipoxygenase gene. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:353-359.

Sinclair,-D.B. 1991. Latent infection of soybean plants and seeds by fungi. Plant Dis. 75:220-224. ill.

Sinclair,-T.R.; Soffes,-A.R.; Hinson,-K.; Albrecht,-S.L.; Pfahler,-P.L. 1991. Genotypic variation in soybean nodule number and weight. Crop Sci. 31:301-304.

Singh,-R.J.; Hymowitz,-T. 1991. Identification of four primary trisomics of soybean by pachytene chromosome analysis. J. Hered. 82:75-77.

Singh,-R.J.; Hymowitz,-T. 1991. Cytogenetics of a synthesized allopentaploid (2n=5x=100) in the genus Glycine Willd. Subgenus Glycine. Genome 34:751-756.

Slater,-G.P.; Elmore,-R.W.; Doupnik,-B.L. Jr.; Ferguson,-R.B. 1991. Soybean cultivar yield response to benomyl, nitrogen, phosphorus, and irrigation levels. Agron. J. 83:804-809.

Slavin,-J. 1991. Nutritional benefits of soy protein and soy fiber. J. Am. Diet. Assoc. 91:816-819.

Small,-J.G.; Botha,-F.C.; Pretorius,-J.C.; Hoffman,-E. 1991. Evidence for an ethylene requirement to reduce soaking injury in bean seeds and the beneficial effect of heavy metals. J. Exp. Bot. 42:277-280.

Smelser,-R.B.; Pedigo,-L.P. 1991. Phenology of Cerotoma trifurcata on soybean and alfalfa in central Iowa. Environ. Entomol. Lanham, Md.: Entomological Society of America. 20:514-519.

Smith,-M.A.L.; wagner,-R.E.; Anderson,-J.S.; Spomer,-L.A. 1991. In vitro assay for evaluation of phytophthora rot on soybean taproots. Crop Sci. 31:1364-1366.

Smith,-O.P.; Hunst,-P.L.; Hewings,-A.D.; Stone,-A.L.; Tolin,-S.A.; Damsteegt,-V.D. 1991. Identification of dsRNAs associated with soybean dwarf virus-infected soybean. Phytopathology 81:131-134.

Spurgeon,-D.W.; Mueller,-A.J. 1991. Sampling methods and spatial distribution patterns for threecornered alfalfa hopper nymphs (Homoptera: Membracidae) on soybean. J. Econ. Entomol. 84:1108-1116.

Staswick,-P.E.; Huang,-J.F.; Rhee,-Y. 1991. Nitrogen and methyl jasmonate induction of soybean vegetative storage protein genes. Plant Physiol. 96:130-136.

Stephens,-P.A.; Barwale-Zehr,-U.B.; Nickell,-C.D.; Widholm,-J.M. 1991. A cytoplasmically inherited, wrinkled-leaf mutant in soybean. J. Hered. 82:71-73.

Suganuma,-N.; Kanada,-H.; Yanagimoto,-N. 1991. Detection of polypeptides involved in early stages of nodulation in soybean roots. Plant Cell Physiol. 32:1259-1266.

Summerfield,-R.J.; Roberts,-E.H.; Ellis,-R.H.; Lawn,-R.J. 1991. Towards the reliable prediction of time to flowering in six annual crops. I. The development of simple models for fluctuating field environments. Exp. Agric. 27:11-31.

Surowy,-T.K.; Boyer,-J.S. 1991. Low water potentials affect expression of genes encoding vegetative storage proteins and plasma membrane proton ATPase in soybean. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:251-262. ill.

Suzuka,-I.; Hata,-S.; Matsuoka,-M.; Kosugi,-S.; Hashimoto,-J. 1991. Highly conserved structure of proliferating cell nuclear antigen (DNA polymerase delta auxiliary protein) gene in plants. Eur. J. Biochem. 195:571-575.

Suzuki,-H.; Verma,-D.P.S. 1991. Soybean nodule-specific uricase (nodulin-35) is expressed and assembled into a functional tetrameric holoenzyme in Escherichia coli. Plant Physiol. 95:384-389.

Tai,-H.; McHenry,-L.; Fritz,-P.J.; Furtek,-D.B. 1991. Nucleic acid sequence of a 21 kDa cocoa seed protein with homology to the soybean trypsin inhibitor (Kunitz) family of protease inhibitors. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:913-915.

Tajima,-S.; Ito,-H.; Tanaka,-K.; Nanakado,-T.; Sugimoto,-A.; Kouchi,-H.; Okazaki,-K. 1991. Soybean cotyledons contain a uricase that cross-reacts with antibodies raised against thenodule uricase (nod-35). Plant Cell Physiol. 32:1307-1311.

Thies,-J.E.; Singleton,-P.W.; Bohlool,-B.B.Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Appl. Environ. Microbiol. 57:19-28.

Thomas,-J.F.; Kanchanapoom,-M.L. 1991. Shoot meristem activity during floral transition in Glycine max (L.) Merr. Bot. Gaz. 152:139-147.

Thomison,-P.R.; Thomas,-C.A.; Kenworthy,-W.J. 1991. Tolerant and root-resistant soybean cultivars: reactions to phytophthora rot in inoculum-layer tests. Crop Sci. 31:73-75.

Tinius,-C.N.; Burton,-J.W.; Carger,-T.E., Jr. 1991. Recurrent selection for seed size in soybean: I. Response to selection in replicate populations. Crop Sci. 31:1137-1141.

Tiwari,-S.P.; Bhatnagar,-P.S. 1991. Pod shattering as related to other agronomic attributes in soybean. Trop. Agric. 68:102-103.

Troedson,-R.J.; Ryley,-M.J.; Byth,-D.E.; Irwin,-J.A.G. 1991. Effect of phytophthora root and stem rot on the response of field-grown soybean to saturated soil culture. Aust. J. Agric. Res.42:791-799.

Tylka,-G.L.; Hussey,-R.S.; Roncadori,-R.W. 1991. Interactions of vesicular-arbuscular mycorrhizal fungi, phosphorus, and Heterodera glycines on soybean. J. Nematol. 23:122-133.

Voldeng,-H.D.; Saindon,-G. 1991. Registration of a high-protein soybean germplasm line OT89-16. Crop Sci. 31:1100.

Voldeng,-H.D.; Saindon,-G. 1991. Registration of four pairs of 'Maple Presto' derived soybean genetic stocks. Crop Sci. 31:1398-1399.

Voldeng,-H.D.; Saindon,-G. 1991. Registration of seven long-daylength insensitive soybean genetic stocks. Crop Sci. 31:1399.

Wagner,-R.E.; Wilkinson,-H.T. 1991. Detoxification and evaluation of foam supports for aeroponically grown soybean. Crop Sci. 31:1071-1073.

Waller,-R.S.; Nickell,-C.D.; Drzycimski,-D.L.; Miller,-J.E. 1991. Genetic analysis of the inheritance of brown stem rot resistance in the soybean cultivar Asgrow A3733. J. Hered. 82:412-417.

Wang,-H.; Cutler,-A.J.; Fowke,-L.C. 1991. Microtubule organization in cultured soybean and black spruce cells: interphase--mitosis transition and spindle morphology. Protoplasma. 162:46-54.

Weaver,-C.D.; Crombie,-B.; Stacey,-G.; Roberts,-D.M. 1991. Calcium-dependent phosphorylation of symbiosome membrane proteins from nitrogen-fixing soybean nodules. Evidence for phosphorylation of nodulin-26. Plant Physiol. 95:222-227.

Webb,-J.; Sheehy,-J.E. 1991. Legume nodule morphology with regard to oxygen diffusion and nitrogen fixation. Ann. Bot. 67:77-83. ill.

Welle,-R.; Grisebach,-H. 1991. Properties and solubilization of the prenyltransferase of isoflavonoid phytoalexin biosynthesis in soybean. Phytochemistry 30:479-484.

Welle,-R.; Schroder,-G.; Schiltz,-E.; Grisebach,-H.; Schroder,-J. 1991. Induced plant responses to pathogen attack. Analysis and heterologous expression of the key enzyme in the biosynthesis of phytoalexins in soybean (Glycine max L. Merr. cHarosoy 63). Eur. J. Biochem. 196:423-430. ill.

Werner,-M.H.; Wemmer,-D.E. 1991. 1H assignments and secondary structure determination of the soybean trypsin/chymotrypsin Bowman-Birk inhibitor. Biochemistry 30:3356-3364.

Wettlaufer,-S.H.; Obendorf,-F.L. 1991. Ureides and amides as nitrogen sources for soybean seed growth and maturation in vitro. Crop Sci.1319-1323.

Whalen,-M.C.; Innes,-R.W.; Bent,-A.F.; Staskawicz,-B.J. 1991. Identification of Pseudomonas syringae pathogens of Arabidopsis and a bacterial locus determining avirulence on both Arabidopsis and soybean. Plant Cell 3:49-59.

Wheeler,-G.S.; Slansky,-F.-Jr. 1991. Effect of constitutive and herbivore-induced extractables from susceptible and resistant soybean foliage on nonpest and pest noctuid caterpillars. J. Econ. Entomol. 84:1068-1079.

Wheeler,-R.M.; Mackowiak,-C.L.; Sager,-J.C. 1991. Soybean stem growth under high-pressure sodium with supplemental blue lighting. Agron. J. 83:903-905.

Wiedermann,-L.; Erickson,-D. 1991. Soybean oil: modern processing and utilization. Int. News Fats Oils Relat. Mater. 2:200-208.

Wilcox,-J.R.; Abney,-T.S. 1991. Inheritance of a narrow, rugose-leaf mutant in Glycine max. J. Hered. 82:421-423.

Wiles,-L.J.; Wilkerson,-G.G. 1991. Modeling competition for light between soybean and broadleaf weeds. Agric. Syst. 35:37-51.

Wilkerson,-G.G.; Modena,-S.A.; Coble,-H.D. 1991. HERB: decision model for postemergence weed control in soybean. Agron. J. 83:413-417.

Williams,-D.F.; Banks,-W.A.; Vander-Meer,-R.K.; Lofgren,-C.S. 1991. Red imported fire ant (Hymenoptera: Formicidae) control with a corn grit bait of fenoxycarb without soybean oil. J. Econ. Entomol. 84:814-817.

Wohlt,-J.E.; Chmiel,-S.L.; Zajac,-P.K.; Backer,-L.; Blethen,-D.B.; Evans,-J.L. 1991. Dry matter intake, milk yield and composition, and nitrogen use in Holstein cows feed soybean, fish, or corn gluten meals. J. Dairy Sci. 74:1641-1647.

Woodruff,-J.M.; Lee,-D.; Thompson,-S.S. 1991. Soybean varieties for Georgia. Bull. Coop. Ext. Serv. Univ. Ga. Coll. Agric. (942,rev.) 8 p.

Wyss,-P.; Boller,-T.; Wiemken,-A. 1991. Phytoalexin response is elicited by a pathogen (Rhizoctonia solani) but not by a mycorrhizal fungus (Glomus mosseae) in soybean roots. Experientia. 47:395-399.

Xue,-Z.T.; Larsen,-K.; Jochimsen,-B.U. 1991. Oxygen regulation of uricase and sucrose synthase synthesis in soybean callus tissue is exerted at the mRNA level. Plant Mol. Biol. Int. J. Fund. Res. Genet. Eng. 16:899-906.

Yanagisawa,-T.; Tano,-S.; Fukui,-K.; Harada,-K. 1991. Marker chromosomes commonly observed in the genus Glycine. Theor. Appl. Genet. 81:606-612.

Yang,-X.B.; Tschanz, -A.t.; Dowler, -W.M.; Wang, -T.C. 1991. Development of yield loss models in relation to reductions of components of soybean invected with Phakopsora pachyrhizi. Phytopathology 81:1420-1426.

Yang,-X.B.; Snow,-J.P.; Berggren,-G.T. 1991. Patterns of Rhizoctonia foliar blight on soybean and effect of aggregation on disease development. Phytopathology 81:287-292.

Ye,-Z.H.; Varner,-J.E. 1991. Tissue-specific expression of cell wall proteins in developing soybean tissues. Plant Cell 3:23-37.

Yen,-J.T.; Cromwell,-G.L.; Allee,-G.L.; Calvert,-C.C.; Crenshaw,-T.D.; Miller,-E.R. 1991. Value of raw soybeans and soybean oil supplementation in sow gestation and lactation diets: a cooperative study. J. Anim. Sci. 69:656-663.

Young,-J.P.W.; Downer,-H.L.; Eardly,-B.D. 1991. Phylogeny of the phototrophic rhizobium strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment. J. Bacteriol. 173:2271-2277.

Zachariassen,-J.A.; Power,-J.F. 1991. Growth rate and water use by legume species at three soil temperatures. Agron. J. 83:408-413.

Zhang, Y.; Parsons,C.M.; Hymowitz, T. 1991. Effect of soybeans varying in trypsin inhibitor content on performance of laying hens. Poultry Sci. 70:2210-2213.

Zhu,-Y.; Schubert,-K.R.; Kohl,-D.H. 1991. Physiological responses of soybean plants grown in a nitrogen-free or energy limited environment. Plant Physiol. 96:305-309.

CURRENT SUBSCRIBERS

GARY R. ABLETT
FARM CROPS SECTION
RIDGETOWN COLLEGE OF AGRIC
RIDGETOWN ONTARIO NOP-2GO
CANADA

LIBRARY
AGRIC RES & ADVISORY STN
PMB GRAFTON NSW
2460
AUSTRALIA

AGRIGENETICS CROP
INFO REC CTR
5649 E BUCKEYE RD
MADISON, WI 53716

MARC ALBERTSEN
PIONEER HI-BRED INTL
7250 NW 62ND AVE/PO BOX 1004
JOHNSTON, IA 50131

SC ANAND
DELTA RESEARCH CENTER
UNIV. OF MISSOURI
PO BOX 160
PORTAGEVILLE, MO 63873

LIBRARY
AQUISITION DEPT.,SERIAL SECTION
UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602

ALAN ATHERLY
ZOOL/GENETICS DEPT
IOWA STATE UNIVERSITY
AMES, IA 50011-1050

JIM BAUMER
LAND-O-LAKES RES FARM
RR 2
WEBSTER CITY, IA 50595

DONN BEIGHLEY
JACOB HARTZ SEED CO
N PARK AVE
BOX 946
STUTTGART, AR 72160

H. ROGER BOERMA
3111 PLANT SCIENCES BLDG
UNIV OF GEORGIA
ATHENS, GA 30602

G. R. BOWERS
TEXAS A&M AGRICULTURE
RT 7 BOX 999
BEAUMONT, TX 77713

DR. H. R. BRADNER
KING AGRO INC
BOX 1088
CHATHAM ONTARIO
CANADA N7M-5L6

A. H. D. BROWN
CSIRO DIV OF PLANT IND
GPO BOX 1600
CANBERRA ACT 2601
AUSTRALIA

EDDIE BROWN
HARTZ SEED CO
PO BOX 946
STUTTGART, AR 72160

DR. G. R. BUSS
CSES DEPT.
VIRGINIA POLYTECH INST STATE
UNIVERSITY
BLACKSBURG, VA 24061-0404

R. I. BUZZELL
RESEARCH STATION
HARROW ONTARIO
CANADA NOR-1GO

ACCESSIONS UNIT CAB
INTERNATIONAL
PO BOX 100
WALLINGFORD OXON
OX10 8DF
ENGLAND

DR. SILVIA CIANZIO
SUBESTACION DE ISABELA
APARTADO 506
ISABELA, PR 00662

DR. RICHARD L. COOPER
DEPT OF AGRONOMY
OHIO AGR RES & DEV CENTER
WOOSTER, OH 44691

BRUCE COULOMBE
PLANT SCI RES INC
PO BOX 2438
W LAFAYETTE, IN 47906

WAYNE CROOK
PIONEER HI-BRED INTERNATIONAL INC
BOX 627
MARSHALL, MO 65340

KATHLEEN DANNA
CAMPUS 347-MCDB
UNIV OF COLORADO
BOULDER, CO 80309-0347

M. R. DAVEY
DEPT OF LIFE SCIENCE
UNIV OF NOTTINGHAM
UNIVERSITY PARK
NOTTINGHAM NG7 2RD
UNITED KINGDOM

T. E. DEVINE
PLANT MOLE GENET LAB
USDA BARC WEST
BLDG 006, RM 118
BELTSVILLE, MD 20705

RICHARD W. DOUGHERTY
HARTZ SEED CO
PO BOX 946
STUTTGART, AR 72160

DEBORAH DRZYCIMSKI
ASGROW SEED CO
634 E LINCOLN WAY
AMES, IA 50010

WILLIAM H. EBY
MIDWEST OILSEEDS INC
2225 LAREDO TRAIL
ADEL, IA 50003

WAYNE ELLINGSON
AGRIPRO SEEDS
RR2 HWY 30 E
AMES, IA 50010

TIMOTHY C. FOLEY
JACQUES SEED CO
RR 2 BOX 84
LINCOLN, IL 62656

RON FRANK
LIFE SCI
UNIV MISSOURI-ROLLA
ROLLA, MO 65401-0249

SCOTT FRANK
SOYBEAN RES FNDN
115 N PERRY ST
MASON CITY, IL 62664

ROBERT FREESTONE
PIONEER HI-BRED INTL
3261 W AIRLINE HWY
WATERLOO, IA 50703

GEORGE GRAEF
319 KEIM HALL
UNIV OF NEBRASKA-LINCOLN
LINCOLN, NE 68583-0915

P. M. GRESSHOFF
PLANT MOLE GENET (OHLD)
269 ELLINGTON PLANT SCI
PO BOX 1071
KNOXVILLE, TN 37701-1071

EARL T. GRITTON
DEPT OF AGRONOMY
MOORE HALL RM 442
UNIV. OF WISCONSIN
MADISION, WI 53706

JAY HACKNEY
145 BAS RIVIERE-NORD
ST. CESAIRE
QUEBEC JOL ITO CANADA

JAMES HARPER
331 PLANT/ANIMAL BIOTECH
1201 W, GREGORY DRIVE
URBANA, IL 61801

KOJI HASHIMOTO
HOKKAIDO NATL AGRIC EXP STN
MEMURO
HOKKAIDO 082 JAPAN

DR. JOHN D. HICKS JR
PIONEER HI-BRED INTERNATIONAL INC
PO BOX 4428
SEVEN OAKS RD
GREENVILLE, MS 38704

KUELL HINSON
IFAS BUILDING 63
UNIVERSITY OF FLORIDA
GAINESVILLE, FL 32611

TED HYMOWITZ
AW-115 TURNER HALL
UNIV OF ILLINOIS
1102 S GOODWIN AVE
URBANA, IL 61801

JUN INOUYE
INST OF TROP AGRIC
KYUSHU UNIV
HAKOZAKI HIGASHI-KU
FUKUOKA 812
JAPAN

JAGMOHAN JOSHI
SOY RES INST
DEPT AGRIC
UNIV MARYLAND EASTERN SHORE
PRINCESS ANNE, MD 21853

JOE C. KAMALAY
DEPT OF AGRONOMY
2021 COFFEY RD #202
COLUMBUS, OH 43210-1086

MICHAEL M. KENTZ
USDA/ARS
PO BOX 196
STONEVILLE, MS 38776

WM J. KENWORTHY
DEPT OF AGRONOMY
UNIV OF MARYLAND
COLLEGE PARK, MD 20742

YUN TZU KIANG
DEPT F PLANT BIOLOGY
NESMITH HALL
UNIV OF NEW HAMPSHIRE
DURHAM, NH 03824-3597

T.C. KILEN
DELTA BRANCH EXPERIMENT STATION
PO BOX 196
STONEVILLE, MS 38776

JIN-KEY KIM
DEPT OF AGRONOMY
COLLEGE OF AGRIC
CHONBAK NATL
UNIV CHONJU 560-756 KOREA

SEOK-DONG KIM
UPLAND CROP DIV
CROP EXP STN RDA
209 SOTUN-DONG KWON SON KU
SUWON 440-100 REP OF KOREA

A. D. KNAPP
AGRONOMY G208
IOWA STATE UNIVERSITY
AMES, IA 50011-3228

PAUL D. KOELLING
PIONEER HI-BRED INTERNATIONAL INC
3261 W AIRLINE HWY
WATERLOO, IA 50703-9610

J. KONOVSKY
EAST-WEST SEED CO
1023 S ADAMS
SUITE 1183
OLYMPIA, WA 98501

Y. C. KWON
DEPT AGRON
GYEONGSANG NATL UNIV
CHINJU 660-701
KYUNGMAN KOREA

R. MARK LAWSON
DEKALB-PFIZER GENETICS
3100 SYCAMORE ROAD
DEKALB, IL 60115

HONG SUK LEE
DEPT OF AGRON
COLLEGE OF AGRIC
SEOUL NATL UNIV
SUWON 441-744 KOREA

ROBERT C. LEFFEL
BLDG 011
HH19
BARC-WEST
BELTSVILLE, MD 20705

M. S. LIN
DEPT OF BOTANY
NATL CHUNG-HSING UNIV
TAICHUNG
TIAWAN 40227

DAVID LOHNES
2303 S FIRST #103
CHAMPAIGN, IL 61820

JEFF LUDLOW
STEWART SEEDS INC
RR 8 PO BOX 227
GREENSBURG, IN 47240

ROGER L. MCBROOM
NORTHRUP KING COMPANY
306 MEADOW DRIVE
BOX Z
ST JOSEPH, IL 61873

L. L. MCCALL
FFR COOP
RR 1 BOX 78
BELLS, TN 38006

BARBARA MCMULLAN
RR 2 PO BOX 411
AGRIPRO BIO SCIENCES INC
BROOKSTON, IN 47923

TADESSE MEBRAHTU
PO BOX 318
VSU
PETERSBURG, VA 23803

A. I. MOHAMED
10130 POST HORN DR
RICHMOND, VA 23237

NATL AGRICULTURAL LIBRARY
SERIALS UNIT-RM 002-VC-07992
USDA
BELTSVILLE, MD 20705

JERZY NAWRACALA
ACAD AGRIC
DEPT OF GENETICS & PLANT BREEDING
60-625 POZAN
UL WOJSKA POLSKIEGO 71 C
POLAND

ERWIN MUELLER
PIONEER HI-BRED INTL
FLIPPEN LANE BOX 649
UNION CITY, TN 38261

R. L. NELSON
USDA/ARS DEPT OF AGRON
UNIV OF ILLINOIS
1102 S GOODWIN
URBANA, IL 61801

JOHN NGUYEN
2364 ALTADENA AVE
SAN DIEGO, CA 92105

CECIL D. NICKELL
DEPT OF AGRONOMY
1102 S GOODWIN AVE.
S-308 TURNER HALL
UNIV OF ILLINOIS
URBANA, IL 61801

AKINORI OKABE
TOHOKU NATL AGRIC EXP STATION
KARIWANO NISHISENBOKU
SENBOKU AKITA 019-21
JAPAN

A. OLIVERI
UNIV DEGLI STUDI DI UDINE
ISTITUTO DI PRODVZIONE VEGETALE
VIA FAGAGNA 208
33100 UDINE ITALY

REID G. PALMER
G301 AGRONOMY
IOWA STATE UNIVERSITY
AMES, IA 50011-1050

PAOLO PARRINI
INST DE AGRONOMIE
35131 PADOVA
VIA GRADENICO 6
ITALY

WAYNE PARROTT
DEPT OF AGRON
COLLEGE OF AGRICULTURE
UNIV OF GEORGIA
ATHENS, GA 30602

DR. EH PASCHAL II
ASGROW SEED COMPANY
205 N MICHIGAN AVE
RT BOX 22
OXFORD, IN 47971

RESEARCH LIBRARY
PIONEER HI-BRED INTL INC
7250 NW 62ND AVE / PO BOX 1004
JOHNSTON, IA 50131

PLANT SCI RES INC
10320 BREN RD EAST
ATTEN: LIBRARY
MINNETONKA, MN 55343

M. RANGAPPA
BOX 453
VIRGINIA STATE UNIV
PETERSBURG, VA 23803

P. RAO-ARELLI
117 CURTIS HALL
UNIVERSITY OF MISSOURI
COLUMBIA, MO 65211

PAUL F. REESE JR.
TIDEWATER AGRIC EXP STN
VPI&SU
PO BOX 7219
SUFFOLK, VA 23437-0219

JOHN ROMBACH
UNITED AGRI SEEDS
PO BOX 4011
CHAMPAIGN, IL 61820-4011

I. A. ROSE
NSW DEPARTMENT OF AGRICULTURE
RESEARCH STATION PMB
MYALL VALE
NARRABRI NSW 2390
AUSTRALIA

DR. J. L. ROSE
DEPT OF PRIMARY INDUSTRIES
HERMITAGE RESEARCH STATION
VIA WARWICK
QUEENSLAND 4370
AUSTRALIA

TAKASHI SANBUICHI
HOKKAIDO CENTER AGRIC
HIGASHI 6
KITA 15 NAGANUMA CHO
YUBARI GUN, HOKKAIDO
JAPAN

V. T. SAPRA
PLANT BREEDING DEPT
PO BOX 67
ALABAMA A&M UNIV
NORMAL, AL 35762

SOUHEI SAWADA
OBIHIRO UNIVERSITY AGRIC & VET
MED
INADACHO OBIHIRO HOKKAIDO
080
JAPAN

T. TSUCHIYA
BARLEY GENETICS NEWSLETTER
DEPT OF AGRONOMY
COLORADO STATE UNIVERSITY
FORT COLLINS, CO 80523

PIERRE TURCOTTE
358 PRINCIPAL CP455
LES BUISSONS
QUEBEC GOH -1HO
CANADA

TERUHISA UMEZAKI
LAB OF CROP SCI
FACULTY OF AGRIC
KYUSHU UNIV
HAKOZAKI HIGASHI-KU
FUKUOKA 812
JAPAN

AGRICULTURAL LIBRARY-SERIALS
226 MUMFORD HALL
UNIV OF ILLINOIS
1408 WEST GREGORY DR
URBANA, IL 61801

ROBERT UPCHURCH
DEPT PLANT PATH
NCSU
RALEIGH, NC 27695-7616

ATTN: MARLYS MCGUIRE
ST PAUL CAMPUS LIBRARY
UNIV OF MINNESOTA
1984 BUFORD AVE
ST PAUL, MN 55108

O/F UNIT
NATIONAL AGRIC LIBRARY
RM 002
10301 BALTIMORE BLVD
BELTSVILLE, MD 20705

FRANCISCO DE JESUS VERNETTI
RUA ANDRADE NEVES 3129
AP 201
96020 PELOTAS RS
BRASIL

LILA VODKIN
DEPT OF AGRONOMY
TURNER HALL
UNIV OF ILLINOIS
1102 S GOODWIN AVE
URBANA, IL 61801

TERRY WALKER
IL FOUNDATION SEEDS
PO BOX 722
CHAMPAIGN, IL 61824-0722

ALLEN K WALKER
ASGROW SEED CO
RR 1 1060 E HWY 14
JAYNESVILLE, WI 53545

L. L. WALLING
DEPT OF BOTANY
PLANT SCI
UNIV OF CALIFORNIA
RIVERSIDE, CA 95251

DAVID B. WEAVER
DEPT OF AGRONOMY & SOILS
AUBURN UNIV
202 FUNCHESS HALL
AUBURN, AL 36849-5412

DAVID WEBB
20874DW
PIONEER HI-BRED INTL
PO 1004
7250 NW 62ND AVE
JOHNSTON, IA 50131

J. M. WIDHOLM
TURNER HALL
DEPT OF AGRONOMY
1102 S GOODWIN AVE
UNIV OF ILLINOIS
URBANA, IL 61801

DR. CURTISS WILLIAMS
JACOB HARTZ SEED CO
PO BOX 946
N PARK AVE
STUTTGART, AR 72160

NANCY ANNE SEBERN
DEKALB-PFIZER GENETICS
PO BOX 8
BEAMAN, IA 50609

TUNEO SEDIYAMA
DEPARTMENTO DE FITOTECHNIA
UNIVERSIDADE FEDERAL DE VICOSA
36.570 VICOSA MG
BRAZIL

M. A. SHAKER
33 ALY MAHMOUD ST
APT 3
HELIOPOLIS
CARIO
EGYPT

Y. SHIMAMOTO
FAC OF AGRONOMY
LABORATORY OF INDUSTRIAL CROPS
HOKKAIDO UNIV
SAPPORO 060
JAPAN

EMERSON R. SHIPE
DEPT OF AGRONOMY & SOILS
CLEMSON UNIVERSITY
CLEMSON, SC 29634-0359

GROVER SHANNON
DELTA AND PINE LAND CO
PO BOX 157
SCOTT, MS 38772

RANDY C. SHOEMAKER
DEPT OF AGRONOMY
IOWA STATE UNIVERSITY
G401 AGRONOMY
AMES, IA 50011-1010

R. J. SINGH
TURNER HALL
1102 S GOODWIN AVE
URBANA, IL 61801

KEITH SMITH
AMERICAN SOYBEAN ASSN
PO BOX 419200
ST LOUIS, MO 63017

JOHN SOPER
PIONEER HI-BRED INTL
RR 4 BOX 68
REDWOOD FALLS, MN 56283

JAMES E SPECHT
DEPT OF AGRONOMY
UNIV OF NEBRASKA
309 KIEM HALL, E CAMPUS
LINCOLN, NE 68583-0915

S. K. ST MARTIN
DEPT OF AGRONOMY
OSU
2021 COFFEY RD
COLUMBUS, OH 43210

RYOJI TAKAHASHI
DEPT OF GENETICS FOR LOW TEMP
HOKKAIDO NATL AGRIC EXP STATION
HITSUJIGAOKA, TOYOHIR-KU
SAPPORA 062
JAPAN

CHRISTOPHER TINIUS
ASGROW SEED CO
PO BOX 210
MARION, AR 72364

DAVID PATTERSON
LAKESIDE BIOTECHNOLOGY INC
2201 W CAMPBELL PARK DR
CHICAGO, IL 60612

TODD PFEIFFER
DEPT OF AGRONOMY
N106 AGRIC SCI BLDG NORTH
UNIV OF KENTUCKY
LEXINGTON, KY 40546-0091

CALLAHAN ENTERPRISES, INC
CLEM WEIDENBENNER
4640 EAST S R 32
LEBANON, IN 46052

LARRY BEACH
PIONEER HI-BRED INTL
7300 NW 62ND AVE
PO BOX 38
JOHNSTON, IA 50131

MADAN KUMAR BHATTACHARYYA
PLANT BIOLOGY DIVISION, NOBLE FOUNDATION
PO BOX 2180
ARDMORE, OK 73402

CAROLYN DE LUNA
AGRACETUS
850 UNIVERSITY GREEN
MIDDLETON, WI 53562

TERRY GRAHAM
DEPT OF PLANT PATHOLOGY
OHIO STATE UNIVERSITY
201 KOTTMAN HALL
2021 COFFEY RD
COLUMBUS, OH 43210

SHARMAN D O'NEILL
DEPT OF BOTANY
ROBBINS HALL
UNIVERSITY OF CALIFORNIA
DAVIS, CA 95616

DOROTHY L. SMITH
BRANDYWINE SEEDS
PO BOX 181
ST. BETHLEHEM, TN 37135

ROD GREDER
ASGROW COMPANY
634 E LINCOLN WAY
AMES, IA 50010

R. FIORITTO
DEPT OF AGRONOMY OARDC/OSU
WOOSTER, OH 44691

STEVE ROBINSON
FFR COOPERATIVE
PO BOX 322
BATTLE GROUND, IN 47920

ALEJANDRO HERNANDEZ
FIRST LINE SEEDS LTD
RR NO 2-GUELPH-ONTARIO
CANADA NIH 6H8

PERIODICAL DEPARTMENT
BEIJING BOOK CO INC
SUB NO 660 B81
701 E LINDEN AVE
LINDEN, NJ 07036-2495

LIBRARY, KARIWANO BRANCH
TOHOKU NATL AGRIC EXP STU
297 KARIWANO, NISHISENBOKU
SENBOKU, ATIKA
019-21 JAPAN

SALLY MACKENZIE
DEPT AGRONOMY
PURDUE UNIV
W LAYFAYETTE, IN 47906

PATRICK PACE
201 FUNCHESS HALL
AUBURN UNIVERISTY
AUBURN, AL 36849-5412

FAHIMA ELHALWAGY
ALLELIX CROP TECHNOLOGIES
PIONEER HI-BRED LIMITED
RESEARCH FARM RR4
GEORGETOWN, ONT
CANADA L7G 4S7

BRIAN DIERS
DEPARTMENT OF CROP AND SOIL SCIENCES
PLANT AND SOIL SCIENCES BUILDING
MICHIGAN STATE UNIVERSITY
EAST LANSING, MICHIGAN 48824

DR. JAMES E. MILLER
PIONEER HI-BRED INTERNATIONAL
7301 NW 62ND AVENUE, PO BOX 85
JOHNSTON, IA 50131-0085

ALFREDO LAM-SANCHEZ
PLANT SCI DEPT
SCHOOL OF AGRIC & VET SCI
STATE UNIV OF SAO PAULO
14870 JABOTICABAL SP
BRAZIL

JOHN VAN HERK JR.
HYLAND SEEDS
PO BOX 130
BLENHEIM ONTARIO
CANADA NOP 1A0

TERESA HARPER
1006 S DAYTON LOT 46
AMES, IA 50010

ASGROW SEED CO
RT 1 BOX 191 A
REDWOOD FALLS, MN 56283
ATTN: ROGER LUSSENDEN

KIM SEOK DONG
LEADER, SOYBEAN BREEDING PROGRAM
CROP EXPERIMENT STATION, RDA
209 SOTUN-DONG KWON SON KU
SUWON 441-100
REPUBLIC OF KOREA

AN LIN DING
INST OF COOP BUILDING OF CULTIVATION CAAS
BEIJING 100081 CHIANA
THAILAND

DREW IVERS
LAND O'LAKES, INC
RR 2
WEBSTER CITY, IA 50592

KRISTI HARKINS
DEPT OF BIOCHEMISTRY & BIOPHYSICS
1117 MOLECULAR BIOLOGY BUILDING
IOWA STATE UNIVERSITY
AMES, IA 50011

LISA LORENZEN
GENETICS
DEPARTMENT OF AGRONOMY
IOWA STATE UNIVERSITY
AMES, IA 50011

FRANCISCO MANDL
INIA LA ESTANZUELA
70006-COLONIA
URUGUAY

BASIL NIKOLAU
DEPT OF BIOCHEMISTRY &
BIOPHYSICS
MOLECULAR BIOLOGY BUILDING
IOWA STATE UNIVERSITY
AMES, IA 50011

GARY POMERANKE
AGRIGENETICS CO
5649 E BUCKEYE RD
MADISON, WI 53716

ANTONI J. RAFALSKI
DUPONT AG BIOTECH
PO BOX E402 EXT STA
WILMINGTON, DE 19880

RHONDA L. MILLER
75 SOUTH 600 EAST
HYRUM, UT 84319

JOE POLACCO
BIOCHEM DEPT
117 SCHWEITZER HALL
UNIVERSITY OF MISSOURI
COLUMBIA, MO 65211

DEAN LEE RESEARCH STATION
LOUISIANA ACRICULTURAL
EXPERIMENT STATION
LOUISIANA STATE UNIVERSITY
AGRICULTURAL CENTER
8105 EAST CAMPUS AVENUE
ALEXANDRIA, LA 71302-9608

NEIL R. REESE
BIOL/MICRO DEPT
BOX 2207 B
SOUTH DAKOTA STATE UNIV
BROOKINGS, SD 57007

RICHARD RUFF
PLANT PATHOLOGY
319 BESSEY HALL
IOWA STATE UNIVERSITY
AMES, IA 50011

DR. RONALD E. SECRIST
DAIRYLAND SEED COMPANY, INC
RR 2 BOX 60A
GIBSON CITY, IL 60936

CLAY SNELLER
115 PLANT SCIENCE
DEPT OF AGRONOMY
UNIVERSITY OF ARKANSAS
FAYETTEVILL, AR 72701

VIRGIL SPARKS
RT 2 BOX 92A
CARROL, IA 51401

TOM THOMPSON
RR 1 BOX 34
LELAND, IA 50453

HARRY H. STINE
MIDWEST OILSEEDS INC
2225 LAREDO TRAIL
ADEL, IA 50003

ANNA TAN-WILSON
DEPT OF BIO SCIENCES
SUNY BINGHAMTON
BINGHAMTON, NY 13901

HERBERT J. SCHMIDT
PIONEER HI-BRED INT'L, INC
R617 CR13A
NAPOLEON, OH 43545

G. L. TYLKA
PLANT PATHOLOGY
351 BESSEY HALL
IOWA STATE UNIVERSITY
AMES, IA 50011

JEFF TYLER
RESEARCH GENETICIST
SOYBEAN PRODUCTION RESEARCH
STONEVILLE, MS 38776

BOB UNIATOWSKI
DEPT OF PLANT & SOIL SCIENCES
U OF DELAWARE
NEWARK, DE 19717-1303

KAN WANG
ICI SEED INC
BOX 500
SLATER, IA 50244

NEVIN DALE YOUNG
UNIVERSITY OF MINNESOTA
495 BORLAUG HALL
ST PAUL, MN 55108

J. H. ORF
DEPT AGRONOMY & PLANT GENETICS
UNIV MINNESOTA
ST PAUL, MN 55108

DR. TARA VAN TOAI
USDA ARS MWA
SOIL DRAINAGE RES UNIT
590 WOODY HAYES DRIVE
COLUMBUS, OH 43210

ELIZABETH GRABAU
DEPT PLANT PATH PHYS WEED
UPI
BLACKSBURG, VIRGINIA 24061

KEITH NEWHOUSE
ICI SEEDS, INC
BOX 500
SLATER, IA 50244

HALINA SKORUPSKA
CLEMSON UNIVERSITY
277 POOLE AGNC CENTER
CLEMSON, SC 29634

M. BALTAZAR
PIONEER HI-BRED INTL
7301 NW 62ND AVE/ PO BOX 1004
JOHNSTON, IA 50131-9900

HOLLY JESSEN
PIONEER HI-BRED INTL
7250 NW 62ND AVE/ PO BOX 1004
JOHNSTON, IA 50131-9900

UNIVERSITY OF ILLINOIS at
URBANA-CHAMPAIGN
111 LIBRARY
1408 W. GREGORY DRIVE
URBANA, IL 61801

P.B. CREGAN
SOYBEAN AND ALFALFA RESEARCH
LAB
RANG 1, HH19 BARC-WEST
BELTSVILLE, MD 20705

JEFF P. TOMKINS
277 POOLE AG SCI
AGRONOMY, CLEMSON UNIV
CLEMSON, SC 29634

ISAO MATSUKAWA
HOKKAIDO CENTRAL AGR EXP STN
NAGANUMA-CHO
YUBARI-GUA
HOKKAIDO 069-13 JAPAN

THOMAS E. CARTER, JR.
USDA/ARS
3127 LIGON ST
BOX 7631
RALEIGH, NC 27607

JOHN GRACE
7301 NW 62nd AVE
PO BOX 85
JOHNSTON, IA 50131-0085

ALBERTO SOLDATI
ETH-INST. PLANT SCIENCES
ESCHIKOU 33
CH-8315
LINDEN
SWITZERLAND

SUK-HA LEE
UPLAND CROP DIVISION I
CROP EXPERIMENT STATION, RDA
SUWON 441-100
KOREA

HOWARD GABE
NORTHRUP KING CO
PO BOX 729
HWY 158E
BAY, AR 72411

DR. JOE BURTON
USDA-ARS
3127 LIGON ST
BOX 7631
RALEIGH, NC 27607-7631

US PATENT & TRADEMARK OFFICE
PC41PAP200173
SCIENTIFIC LIBRARY
CP3-2C06
WASHINGTON, DC 20231